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MICROBIOLOGICAL ANALYSIS OF SOIL AS AN INDEX OF SOIL FERTILITY: VIII. DECOMPOSITION OF CELLULOSE[1]

SELMAN A. WAKSMAN AND O. HEUKELEKIAN

New Jersey Agricultural Experiment Stations

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Among the constituents of plant residues, green manures and stable manure added to the soil, cellulose occupies an important place. A study of the organisms concerned in the decomposition of cellulose, including bacteria, fungi and actinomycetes, has occupied the attention of many investigators and any new contribution to this subject has always aroused great interest. As a matter of fact it has not been definitely established as yet just how far celluloses are broken down by microörganisms, the chemistry of the process being in most cases, still an open question. Until the development of Charpentier's (3) method, there was no adequate method for the quantitative determination of cellulose in the soil. Most of the studies on cellulose decomposition in the soil by microörganisms have been limited to qualitative results, such as the mechanical disintegration of filter paper, formation of pigments and growth of organisms on the paper, formation of clear zones around the colonies on cellulose agar, gas formation, etc.

By means of an accurate quantitative method it is possible to study the rapidity of cellulose decomposition in different soils, as influenced by soil type, physical and chemical condition, micro-flora, etc.

Christensen (4) was the first to suggest that the power of a soil to decompose cellulose may serve as an index of soil fertility. A definite amount of the soil to be investigated was placed in Erlenmeyer flasks so as to cover four-fifths of the bottom of the flasks. The water was added from a pipette to the uncovered part of the bottom of the flask. A few strips of filter paper were then pressed upon the soil, which was kept moist all the time. From nine to ninety-three days were required for the complete decomposition of the paper, depending upon the soil. Christensen (5) has further shown that the physical condition of the soil as well as its reaction do not influence greatly its cellulose-decomposing capacity. The presence of available minerals, primarily phosphates, as well as available nitrogen, are of first importance, and, in some cases, also the microbial flora; in other words the phenomenon of cellulose decomposition is influenced by the chemical and microbiological soil conditions. The amount of cellulose decomposed is governed by the available nitrogen and phosphates in the soil. In general, the chemical composition of the soil was found to be of much greater importance to the cellulose decomposition than the microbial flora. Only in the case of certain peat soils, did the inoculation with cellulose-decomposing bacteria have any effect. By the use of Christensen's method, Arnd (1) found that fertilized, limed and cultivated peat soils possessed a

[1] Paper No. 157 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

greater cellulose-decomposing power than untreated peat soil; the soil treated with stable manure was most active.

Mütterlein (7) criticised Christensen's method as allowing the development of only aerobic organisms and not allowing for the influence of moisture, and other soil conditions. He suggested the placing of one or two pieces of filter paper of a uniform weight, 10–20 gm., at various depths of soil, then weighing the residue, by removing the paper from the soil after two or three weeks.

Niklewski (8) added cellulose to the soil and determined the evolution of carbon dioxide as a result of the decomposition of cellulose. In addition to cellulose, 1 gm. K_2HPO_4, 1 gm. $MgSO_4$, 8 gm. $CaCO_3$ and $(NH_4)_2SO_4$ were added to 8 kgm. of soil. He found that the decomposition of cellulose in the soil is chiefly controlled by the presence of available nitrogen. The greater the amount of cellulose added to or present in the soil, the quicker will the nitrogen need set in. Nitrogen-fixing organisms were thought to play only a secondary rôle in normal soils, since cellulose is decomposed in those soils, without the addition of available nitrogen, only very slowly; this would not be the case, if nitrogen-fixing organisms were active. When only 0.125 per cent cellulose is added, there is already noticed a nitrogen need in a loess soil containing 0.15 per cent nitrogen. The greater the amount of cellulose added, the greater is the evolution of carbon dioxide up to a certain concentration, 1.5 per cent giving at first less carbon dioxide than 1.0 per cent cellulose. When the available nitrogen is lacking the curve soon falls to a certain level depending upon the rapidity of decomposition of the nitrogenous substances in the soil. The addition of 0.0125 per cent ammonium sulfate greatly stimulated cellulose decomposition. For 10 gm. of cellulose 1 gm. of $(NH_4)_2SO_4$ is used in the case of soil and 2 gm. in sand. Larger amounts of nitrogen acted injuriously, this injurious action may be later overcome.

On comparing the evolution of carbon dioxide with and without the addition of a nitrogen salt, Niklewski suggested the calculation of the available nitrogen in the soil; in the case of a loess soil containing 0.150 per cent nitrogen, 0.040 per cent was found to be active, while in the case of a sandy soil with 0.015 per cent, all the nitrogen was active.

Rahn (11) also found that when 0.5 to 1.0 per cent of straw is added to the soil, the available nitrogen is rapidly assimilated by the microörganisms using the straw as a source of energy, and a nitrogen-minimum ensues. When available nitrogen is added, the straw is rapidly transformed into substances with a constant carbon-nitrogen ratio.

Charpentier (3) added 1 per cent of cellulose, in the form of finely divided filter paper, to soil sieved through a 2-mm. sieve, and after carefully mixing the paper with the soil, the proper amount of moisture was introduced. At the end of the incubation period, the soil was air-dried and the amount of cellulose left was determined. Stable manure was found to have a decided stimulating effect upon the decomposition of cellulose in the soil, especially when the moisture content is satisfactory. The influence of reaction is not of great importance in the decomposition of cellulose. The favorable influence of manure is due to the nutrients present, especially the nitrogen. The greater the amount of nutrients (nitrogen and minerals) present in the manure, the greater is its action. The poorer the soil is, the greater is the influence of the manure. When ammonium sulfate and manure containing the same amount of nitrogen are added to the soil, the stimulating effect upon cellulose decomposition was found to be the same. These results were confirmed by Barthel and Bengtsson (2).

A study of the literature on the subject of cellulose decomposition in the soil as an index of microbiological activities would, therefore, lead us to assume that we are dealing here more with a function indicating the physical and chemical condition of the soil, especially the available nitrogen, rather than the specific microbial flora. This can be found to hold true, to some extent at least, after a careful theoretical consideration of the subject.

Cellulose is decomposed in the soil by fungi, actinomycetes and bacteria, including aerobic and anaerobic forms, thermophilic and denitrifying organisms. Probably all soils contain some, if not all, of these groups capable of decomposing cellulose. When cellulose is added to the soil, one of these groups will be favored more than the others, depending upon the physical and chemical condition of the soil, especially reaction, aeration, moisture content, and the presence of available nitrogenous substances. Bacteria which decompose cellulose are incapable of assimilating atmospheric nitrogen and have to depend upon fixed available compounds for their nitrogen supply. Whether nitrogen-fixing bacteria are capable of utilizing the intermediary products of cellulose decomposition, as found by Pringsheim (9) and others, is another point and need not concern us here. It is sufficient to say that cellulose offers a readily available source of energy for organisms capable of utilizing it, and these will do it only in the presence of available nitrogen and mineral salts. It has been found in this laboratory[2] that there is a definite relation between the amount of cellulose decomposed and nitrogen assimilated by an organism, especially in the case of fungi, which were studied most extensively; this ratio was found to be, for these organisms, 30 to 1; i.e., about 30 parts of cellulose are decomposed for every part of nitrogen assimilated, either from ammonium sulfate or sodium nitrate. This ratio will probably be found to be wider for bacteria and actinomycetes, since these organisms produce a relatively much smaller amount of growth than the fungi. In view of this definite relation, the available nitrogen becomes the controlling factor. It remains to be seen whether there is, in normal soils, a different relation between the cellulose decomposition and the amount of nitrogen available, because of the mixed microflora.

METHODS

The determination of cellulose decomposition in the soil can be carried out by two methods: (*a*) Measuring the evolution of carbon dioxide as a result of decomposition of cellulose, as suggested by Niklewski (8), Dvorak (6) and Rahn (10, 11) and as reported in detail elsewhere (16); and (*b*) according to the method of Charpentier (3) and Barthel and Bengtsson (2). The second method is carried out as follows:

Preparation of Schweitzer's reagent. Two hundred grams of copper sulfate are dissolved in hot water and precipitated with a calculated amount of ammonia (95 cc. of ammonia, sp. gr. 90). The excess of ammonia is then neutralized with sulfuric acid. The precipitate is washed by decantation in a large bottle three or four times and is then transferred to a Büchner funnel and filtered through hardened filter paper by the use of suction. With the aid of a porcelain spoon the excess of water is pressed out from the copper hydroxide. It is then removed in the form of a hardened paste from the filter paper and introduced into a bottle containing ammonia water and shaken in a shaking machine for 4–5 hours. An undissolved

[2] Results to be published later.

part of the copper hydroxide should remain at the bottom of the flask. The Schweitzer's reagent prepared in this way should contain 1.5 gm. of copper per 100 cc. of solution.

To test the strength of the reagent, 5 cc. are placed in a crucible of constant weight, near a dish of H_2SO_4 under a bell-jar. As soon as all the ammonia is absorbed, the $Cu(OH)_2$ is dried and heated to constant weight and weighed as CuO.

Process of cellulose determination. Cellulose is added to the soil either in the form of finely cut or well ground filter paper. After the soil is properly mixed, a 20-gm. sample is obtained from moist, or preferably air-dried, soil. The sample is placed in a 250-cc. sampling bottle, 100 cc. of the Schweitzer's reagent is added; the bottle is then stoppered with a rubber stopper and shaken for an hour in a shaking machine. After settling, somewhat more than 50 cc. of the liquid is filtered through a Gooch crucible by the use of suction. Fifty cubic centimeters of the filtrate are then precipitated with 200 cc. of 80 per cent alcohol and the precipitate is filtered through a Gooch crucible and washed as follows: (1) dilute 1 per cent HCl, (2) warm distilled water, (3) dilute 2 per cent KOH to get rid of humic acids—washing with KOH is continued until all brown color disappears, (4) warm distilled water, (5) dilute 1 per cent HCl to get rid of free alkali, (6) warm distilled water until free from chlorides, (7) alcohol—after cooling the crucible, (8) ether.

Dry to constant weight at 110° C., weigh, burn off and weigh again. The difference between the two weights gives the quantity of cellulose for 10 gm. of the sample.

Before the cellulose-decomposing power of the soil could be tested and compared with the crop productivity of the soil, some information had to be gained on a proper incubation period to be used, on the influence of air-drying of soil and the addition of available nitrogen upon cellulose decomposition. The temperature of incubation was always 25°–28°C. while the moisture content was 60 per cent of the maximum moisture-holding capacity of the soil. The soil used in the preliminary experiments was a sassafras gravelly loam with a pH value of 6.2 and average fertility characteristic of this series.

The influence of the amount of cellulose added and period of incubation upon the decomposition of cellulose in the soil previously air-dried is shown in table 1. In all cases, the substances extracted by ammoniacal copper solution and precipitated with alcohol were determined in the control soil, to which no cellulose had been added. The weight of these substances was subtracted from the weight of the cellulose determined in the treated soil. The studies were all done in duplicate, and the averages only are given, since a fair check was obtained in the majority of cases.

There is a decided increase in the actual amount of cellulose decomposed with the increase in the amount of cellulose added to the soil. However, the per cent of cellulose decomposed decreases with the increase in the concentration of cellulose, especially when the amount of cellulose added is large. This is particularly true when the cellulose is added directly to fresh soil and not to air-dried soil, which was used in the previous experiment.

Table 2 shows the influence of air-drying and the addition of an available source of nitrogen upon cellulose decomposition in the soil. In this case the same sample of fresh soil was divided into two portions, one-half of which was kept moist and one-half air-dried, for two weeks. At the end of that period of time, 100-gm. portions of the moist and air-dried soils were placed in

tumblers, 1 gm. of finely divided filter paper was added to each, the soil and cellulose were well mixed, the proper amount of moisture added, tumblers covered with glass plates and placed in the incubator for twenty-one days. To some of the tumblers 50 mgm. of ammonium sulfate were added. The results are given in table 2.

Air-drying exerts a decided stimulating effect upon the decomposition of cellulose. This is due to the fact that air-drying followed by moistening of soil brings about a greater availability of the soil nitrogen. In this particular experiment, 100-gm. portions of the air-dry and moist soils were incubated

TABLE 1

Influence of cellulose concentration and period of incubation on cellulose decomposition

CELLULOSE ADDED PER 100 GM. OF SOIL	INCUBATION	CELLULOSE DECOMPOSED	
gm.	*days*	*gm.*	*per cent*
0.25	7	0.089	35.4
	18	0.168	67.1
	32	0.200	80.0
0.50	7	0.141	28.2
	18	0.293	58.6
	32	0.411	82.2
1.00	7	0.230	23.0
	18	0.547	54.7
	32	0.663	66.3

TABLE 2

Influence of air-drying and addition of available nitrogen upon cellulose decomposition in the soil.

SOIL TREATMENT	CELLULOSE DECOMPOSED IN 21 DAYS
	per cent
Fresh moist soil	24.0
Soil air-dried 14 days	34.9
Moist soil + 50 mgm. $(NH_4)_2SO_4$	82.9
Air dry soil + 50 mgm. $(NH_4)_2SO_4$	83.8

separately, without the addition of any cellulose. It was found that, at the end of the 21-day period of incubation, the soil that was previously air-dried contained 2.9 mgm. of inorganic nitrogen (as ammonia and nitrate) more than the soil kept constantly moist. The actual quantities of cellulose decomposed, as reported in table 2, are less than those reported in table 1. This is undoubtedly due to the fact that the soil used in the first experiment (table 1) was air-dried for a considerable period of time and, as shown by Waksman and Starkey (14), the greater the period of air-drying of a soil, the greater is the increase in the decomposition of the soil organic matter, when the soil is moistened, resulting in an increase in available nitrogen.

These results tend to confirm those of Christensen (5), Niklewski (8), Charpentier (3) and Barthel and Bengtsson (2), that the amount of cellulose decomposed in the soil is a result of the concentration of available nitrogen. Even where 50 mgm. $(NH_4)_2SO_4$ has been added to 100 gm. of soil and 1 gm. of filter paper, no ammonia or nitrate nitrogen could be detected at the end of the experiment, showing that all the nitrogen had been assimilated by microorganisms and probably converted into microbial protein.

To learn what groups of organisms are stimulated most by the addition of cellulose to the soil, 1-gm. portions of filter paper were added to 100-gm. portions of three differently treated soils and placed in tumblers; to some of the tumblers, 100 mgm. of $NaNO_3$ was added. The proper amount of moisture was then added to each soil and tumblers covered and incubated for seventeen days. At the end of that period, the fungi, actinomycetes and

TABLE 3

Influence of 1 per cent of cellulose, with and without $NaNO_3$, upon the development of microorganisms in the soil

SOIL NUMBER	TREATMENT	REACTION	$NaNO_3$ ADDED PER 100 GM. OF SOIL	FUNGI		BACTERIA		ACTINOMYCETES	
				No cellulose	Cellulose added	No cellulose	Cellulose added	No cellulose	Cellulose added
		pH	*mgm.*	*thousands*	*thousands*	*thousands*	*thousands*	*thousands*	*thousands*
5A	Manured, unlimed	5.5	0	87.3	320	6,500	21,920	1,800	6,400
		5.5	100	87.3	3,100	6,500	40,400	1,800	4,600
7A	Unmanured, unlimed	5.1	0	115.7	160	3,900	3,600	1,260	600
		5.1	100	115.7	4,800	3,900	2,480	1,260	400
7B	Unmanured, limed	6.5	0	25.4	47	7,700	17,400	2,760	2,200
		6.5	100	25.4	290	7,700	47,000	2,760	3,200

bacteria were determined by the plate method, described elsewhere (12). The results are given in table 3. The amounts of cellulose decomposed in these soils are found in table 9.

The largest amount of cellulose was decomposed in 5A to which nitrogen in the form of $NaNO_3$ was added (818.5 mgm.), followed by 7B + $NaNO_3$ (535.8 mgm.) and 7A + $NaNO_3$ (270.7 mgm.); considerably smaller amounts of cellulose were decomposed when no $NaNO_3$ was added, as shown in table 9 where the results on cellulose decomposition obtained in this experiment are reported.

It is thus found that the addition of cellulose to the soil greatly stimulates the development of fungi and bacteria and to a lesser extent of actinomycetes. The relative stimulation depends, however, on the nature of the soil. In the case of the heavily manured but acid soil (5A), the addition of 1 per cent of cellulose brought about the same relative increase in the numbers of bacteria,

fungi and actinomycetes; the amount of cellulose decomposed, without the addition of available nitrogen, was only about 0.1 of a gram. However, when nitrogen was added, in the form of $NaNO_3$ (100 mgm.), the amount of cellulose decomposed increased six to seven times, the number of fungi increased ten times, the number of bacteria increased two-fold and the number of actinomycetes was even less than where no nitrogen has been added.

In the case of the exhausted acid soil (7A), the addition of cellulose without any available nitrogen brought about a slight increase in the number of fungi, no perceptible change in the number of bacteria and even a decrease in the number of actinomycetes. The amount of cellulose decomposed, as indicated by the evolution of carbon dioxide, was very small. But when 100 mgm. of $NaNO_3$ was added, the number of fungi increased thirty times, while the bacteria and actinomycetes not only did not increase but even decreased. In this case all the cellulose decomposed (270.7 mgm.) was due to the activities of fungi. The fact that no greater amount of cellulose was decomposed in this soil may be due either to the absence, in this soil, of available minerals, notably phosphates, or to the lack of activity of the bacteria and actinomycetes, possibly due to the acidity of the soil (pH = 5.0). The addition of cellulose to the limed unfertilized soil (7B), which is favorable for the development of bacteria and unfavorable for fungi, due to the reaction of the soil (pH = 6.4), brought a slight increase in the number of fungi and an appreciable increase in the number of bacteria; when nitrogen was added, the increases in the numbers of both groups of organisms were even more marked. The actinomycetes were hardly affected.

This experiment indicates that, in slightly acid soils, the fungi may be most concerned with the decomposition of cellulose, while in limed, neutral or slightly acid soils, both fungi and bacteria play an active part. The lack of development of actinomycetes may be due to the short period of incubation used, since these organisms develop only very slowly.

The fungi produce an abundant growth in the soil and require a minimum amount of nitrogen (3–8 per cent) for the synthesis of their protoplasm. In the absence of the available nitrogen, the fungi, as well as the cellulose decomposing actinomycetes and bacteria, will develop only to a limited extent, and, therefore, will decompose only a limited amount of cellulose.

It was shown by Pringsheim (9), Dvorak (5) and others that Azotobacter is capable of fixing nitrogen using cellulose or its decomposition products as a source of energy. This process seems to be a rather slow one, otherwise nitrogen would not become the limiting factor in cellulose decomposition.

A detailed study of the influence of available nitrogen on cellulose decomposition will be reported elsewhere, here we may merely cite a typical experiment (table 4), using 1 gm. of cellulose in 100 gm. of soil and incubating for 6 weeks.

The addition of a small amount of nitrogen stimulated cellulose decomposition in the fertile soil to a greater extent than in the unfertile soil. This again may be due to the difference in available minerals or in the microflora.

When we come to compare the difference in soil fertility, using the power of cellulose decomposition as an index, we must keep in mind the fact that the soils will vary in their nitrogen content and in the rapidity with which the nitrogen becomes available, as indicated by the process of nitrification (13). If two soils vary in the available nitrogen, we would, therefore, expect that, *ipse facto*, they should also vary in the amount of cellulose decomposed, outside of any differences in their microbial flora.

The same series of nitrogen plots that was used in the previous studies reported in this series of papers dealing with the methods of determining the microbiological condition of the soil, were also used in these experiments. Soils 5A and 5B received yearly applications of 32 tons of cow manure and minerals

TABLE 4

The influence of available nitrogen, as $NaNO_3$, upon cellulose decomposition in the soil

$NaNO_3$ PER 100 GM. OF SOIL	CELLULOSE DECOMPOSED	
	Unfertile soil (7A), 6 weeks incubation	Fertile soil (5A), 4 weeks incubation
mgm.	*per cent*	*per cent*
0	36.9	42.2
25	41.7	66.7
100	59.7	97.2

TABLE 5

Cellulose decomposition in soils of different fertility

PLOT NUMBER	SOIL REACTION	NITRATE NITROGEN IN 100 GM. ORIGINAL SOIL	CELLULOSE DECOMPOSED
	pH	*mgm.*	*per cent*
5A	5.4	0.17	64.5
5B	6.7	0.20	72.8
7A	5.0	0.04	31.9
7B	6.4	0.04	50.9
9A	5.6	0.09	55.3
11A	4.4	0.08	52.7

(640 pounds of acid phosphate and 320 pounds of potassium chloride) per acre; soils 7A and 7B received no fertilizer; soils 19A and 19B, minerals only; soil 9A, 320 pounds $NaNO_3$ and minerals; 11A and 11B, $(NH_4)_2SO_4$ equivalent in nitrogen to 320 pounds $NaNO_3$ and minerals. In addition, all the *B* plots received two tons $CaCO_3$ every five years.

The first samples were taken December 6, 1922, when the soil was frozen. After allowing the soil to thaw off, the excess of water was evaporated by exposure to the air for 48 hours, 0.5 per cent of cellulose was added to 100-gm. portions of the soil (on a dry basis,) and the mixed soil was placed in tumblers, covered with glass and incubated for 6 weeks at optimum moisture. The amounts of cellulose decomposed as well as the amounts of nitrate nitrogen originally present in the soil are given in table 5.

The results presented in table 5 show that there is a certain parallelism between the cellulose-decomposing power of differently treated soils and the available nitrogen in those soils, as shown by their nitrate content. No correlation at all is found between the soil reaction and cellulose decomposition. . Similar results were also obtained by Christensen (5), Charpentier (3), and Barthel and Bengtsson (2).

To demonstrate definitely whether the differences obtained in the amounts of cellulose decomposed in the soils taken from plots of different fertility is due to the differences in the nitrogen availability in the particular soils or to the differences in the microbiological flora brought about by the continued treatment of the soil, the plots were again sampled on June 26, 1923. The soils were tested in two ways for their cellulose-decomposing power: (1) by

TABLE 6

*Influence of soil treatment and addition of available nitrogen upon cellulose decomposition in the soil**

PLOT NUMBER	SOIL REACTION	NO_3-N IN 100 GM. OF SOIL†	CELLULOSE DECOMPOSED	
			No nitrogen added	50 mgm. $NaNO_3$ per 100 gm. of soil
	pH	*mgm.*	*per cent*	*per cent*
5A	5.6	1.70	35.5	79.1
5B	6.8	2.90	53.6	77.7
7A	4.8	0.52	13.1	45.7
7B	6.8	1.02	37.2	80.9
11A	4.2	0.75	46.6	88.9
11B	6.2	1.60	36.5	86.6
19A	5.2	0.60	22.4	69.5
19B	6.5	1.80	52.2	85.6

* 1 per cent filter paper (about 94 per cent pure cellulose), period of incubation 30 days.

† Nitrate-nitrogen accumulated in the same soils, incubated under the same conditions, but without the cellulose.

adding cellulose (1 per cent filter paper), with and without available nitrogen, to the soil and (2) by adding a small amount of soil (1 gm.) to 100 gm. of sterile sand, containing 1 gm. of filter paper and the necessary mineral nutrients. The nitrate nitrogen was determined in control soils incubated with the same amount of moisture for the same period of time, but without the addition of cellulose. The results are given in tables 6 and 7.

The results speak for themselves. When no available nitrogen is added to the soil together with the cellulose, there is found a decided difference in the cellulose-decomposing power of fresh soils taken from variously treated plots, in which differences in fertility have been established. The differences correspond, however, to a large extent, to the available nitrogen in the soil as indicated by their nitrate content, with but one exception, namely 11A. This soil which has been receiving yearly applications of minerals and am-

monium sulfate has been made so acid that the nitrifying capacity has been injured. Some of the available nitrogen is probably present in the form of absorbed ammonium compounds which are readily utilized as a source of nitrogen by the cellulose-decomposing microörganisms.

There is no correlation between the reaction of the soil and its cellulose-decomposing capacity, since the fungi which take a very active part in the decomposition of cellulose in the soil will thrive readily even under the most acid reactions in which cultivated plants will barely grow at all.

To test the cellulose-decomposing activities of the microbiological population of the different soils, 1 gm. of the variously treated soils was added to 100 gm. of sterile sand containing 1 per cent of filter paper and 20 cc. of a mineral solution (containing 10 gm. of $(NH_4)_2SO_4$, 3 gm. K_2HPO_4, 2.0 gm. $MgSO \cdot 7H_2O$ and 1.0 gm. NaCl in 1000 cc. of distilled water) and placed in 250-cc. Erlenmeyer flasks; these were incubated for 21 days. The amount of cellulose decomposed was found to be practically the same for all soils, since all of them harbor organisms capable of decomposing cellulose. Under

TABLE 7

Decomposition of cellulose in sand by 1 gm. portions of variously treated soils

PLOT NUMBER	CELLULOSE DECOMPOSED
	per cent
5A	92.8
5B	91.7
7A	94.9
7B	96.1
11A	93.6
11B	92.0

optimum conditions, these organisms will all develop rapidly and decompose the cellulose, as shown in table 7. This method can, therefore, be only qualitative in nature.

To bring out more definitely the influence of addition of available nitrogen and period of incubation upon the amount of cellulose decomposed, another set of samples, taken September 29, 1923, were placed, in 100-gm. portions, in tumblers. One gram of finely cut filter paper was added to each and well mixed with the soil; 100 mgm. of $NaNO_3$ was added to some of the tumblers; the soils were brought to optimum moisture, the tumblers covered and incubated at 25–28°. The results are given in table 8.

The results indicate that a certain differentiation between the cellulose-decomposing capacity of differently treated soils is found, when a long period of incubation (42 days) is used and no available nitrogen is added; when some available nitrogen is added to the soil, a short period (14–15 days) of incubation is required. When no available nitrogen is added, the differences in cellulose decomposition are due to a large extent to the available nitrogen in the soil; when available nitrogen is added, the differences in cellulose de-

composition are either due to the presence or absence of other chemical elements in the soil, such as phosphates, or to differences in the microbiological activities in the different soils.

Niklewski (8) suggested using the evolution of carbon dioxide as an index of cellulose decomposition in the soil. To compare the evolution of carbon dioxide with the decomposition of cellulose as influenced by the available nitrogen and soil microflora, soils 5A, 7A and 7B were used: the first (5A) is heavily manured, has a pH value of 5.5 and is rich in microörganisms; the second (7A) is unfertilized, has a pH value of 5.0–5.1, is rich in fungi and low in bacteria; the third (7B) is unfertilized but limed, has a pH value of 6.5, is rich in bacteria and poor in fungi. One gram of filter paper was added to 100 gm. of soil and well mixed; the soil was then placed in an apparatus described elsewhere (15), the proper amount of moisture was then added, and the evolution of carbon dioxide measured at frequent intervals. After 17

TABLE 8

Influence of period of incubation and presence of available nitrogen on cellulose decomposition

PLOT NUMBER	NO NITROGEN ADDED		100 MGM. $NaNO_3$ ADDED
	Cellulose decomposed		Cellulose decomposed in 14 days
	In 14 days	In 42 days	
	per cent	*per cent*	*per cent*
5A	18.4	33.8	87.8
7A	6.7		30.5
9A	18.7		79.4
19A	18.7	16.2	58.5
5B	16.5	24.9	72.6
7B	11.8	9.2	60.1
11B	16.1		79.4
19B		16.5	64.7

days of incubation, the nitrate and cellulose were determined in the soil. The results are given in table 9. The changes in the number of microörganisms, as a result of addition of cellulose, with and without $NaNO_3$, to these soils, are recorded in table 3.

The results show definitely that both the evolution of carbon dioxide and the determination of residual cellulose can serve as measures of the power of the soil to decompose cellulose and that the decomposition of cellulose may serve to some extent as an index both of the available nitrogen in the soil and of the activities of the microbiological flora. When no soluble nitrogen was added, the amounts of cellulose decomposed run parallel to the amounts of nitrate that will accumulate in the soil; thus serving as an index of the availability of the soil nitrogen. But decided differences are obtained even when sufficient available nitrogen is added. These differences may be due to the differences in the microbiological activities of the different soils, as

pointed out above. Of course we must consider, in addition to the influence of available nitrogen, also the influence of available minerals, especially potassium salts and phosphates, as well as $CaCO_3$ upon the decomposition of cellulose in the soil. To eliminate this factor, the following method, similar to that of Niklewski (8) was finally adopted:

Two hundred milligrams $CaCO_3$, 50 mgm. K_2HPO_4, 25 mgm. $MgSO_4$ and 1 gm. of finely cut filter paper were added to each 100-gm. portion of soil and well mixed in. The soil was then placed in the apparatus used for the evolution of carbon dioxide and enough water added to bring the moisture content to optimum. Two flasks were used for every soil. The amount of CO_2 evolved from the same soils to which the $CaCO_3$ and minerals were added, but not the cellulose, was also determined. The results of a typical experiment are given in table 10.

TABLE 9

*Influence of addition of available nitrogen upon cellulose decomposition in different soils**

SOIL NUMBER	$NaNO_3$ ADDED TO 100 GM. SOIL	NO_3-N* USED UP	EVOLUTION OF CO_2			CELLULOSE DECOMPOSED IN 100 GM. OF SOIL
			In soil + cellulose	In soil alone	Due to addition of cellulose	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm*	*mgm*
5A	0	3.50	282.70	72.97	209.73	109.7
5A	100	17.90	712.80	72.88	639.92	792.5
7A	0	0.95	72.60	22.24	50.36	47.8
7A	100	6.65	171.05	22.24	148.81	189.7
7B	0	1.20	142.45	57.83	84.62	
7B	100	10.50	429.55	58.82	371.73	454.8

* Nitrate formed in soil was determined in separate portions to which no cellulose has been added; nitrate-nitrogen used up was obtained by subtracting the residual nitrates from that formed in the control soil + the added nitrate, per 100 gm. of soil. Period of incubation was 17 days.

TABLE 10

Decomposition of cellulose in various soils as indicated by the evolution of CO_2 and amount of cellulose left

SOIL NUMBER	FINAL REACTION	NITRATE-N ACCUMULATION IN 100 GM. SOIL WITHOUT CELLULOSE	EVOLUTION OF CO_2 IN 21 DAYS			CELLULOSE DECOMPOSED IN 100 GM. OF SOIL
			Soil + cellulose	Soil alone	Due to addition of cellulose	
	pH	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
5A	6.7	2.9	764.10	107.22	656.88	451
5B	6.9	1.4	544.50	67.65	476.85	418
7A	6.5	0.7	540.40	127.05	413.35	310
9A	6.4	1.5	417.80	132.00	285.80	213
11A	6.0	2.3	692.60	156.75	535.85	407
11B	6.8	1.2	424.00	82.50	341.50	210

The addition of $CaCO_3$ and minerals to the soil brings about a greater evolution of carbon dioxide in the acid than in the limed plots (*B*). This is probably partly due to the chemical liberation of carbon dioxide by the

interaction of organic and inorganic substances and $CaCO_3$. It may also be due to improvement in the physical condition of the acid soils by the calcium carbonate and stimulation of activities of bacteria. The greatest amounts of cellulose were decomposed, as shown by the residual cellulose, in the manured soils (5A and 5B), followed closely by the ammonium sulfate soil (11A). Although 11A contained almost twice as much available nitrogen (as nitrate) than 5B, the amount of carbon dioxide produced from 11A was only slightly greater than from 5B, while the total amount of cellulose decomposed was actually smaller. This is probably due to the fact that the fungi which are more active in 11A, require more nitrogen for every unit of cellulose decomposed than the bacteria, which are more active in 5B. The sodium nitrate soil (9A) and limed, ammonium sulfate soil (11B) gave the smallest amounts of cellulose decomposed, both by measuring the residual cellulose and the carbon dioxide evolved, less so than the unfertilized soil 7A. This again points to the fact that the available nitrogen, although a very impor-

TABLE 11

Influence of $NaNO_3$ upon the decomposition of cellulose, in the presence of $CaCO_3$ and minerals

SOIL NUMBER	$NaNO_3$ ADDED TO 100 GM. OF SOIL	EVOLUTION OF CO_2 IN 14 DAYS			CELLULOSE DECOMPOSED IN 100 GM. OF SOIL
		Soil + cellulose	Soil alone	Due to addition of cellulose	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm*
5A	0	544.50	75.50	469.00	387
5A	25	778.25	75.50	702.75	567
5A	50	817.30	75.50	741.80	630
7A	0	364.10	88.60	275.50	222
7A	25	526.35	88.60	437.75	437
7A	50	684.20	88.60	595.60	557

tant factor affecting the amount of cellulose that can be decomposed in a given soil and in the rapidity of decomposition of the cellulose, may not be the only factor concerned. When sufficient calcium carbonate and minerals are added to the soil, the amount of cellulose decomposed can serve in a way as an index of the nitrogen of the soil that can become available, if sufficient lime and minerals are added.

To test this point further, six 100 gm. portions of soil 5A and of soil 7A were placed in the respiratory flasks; 200 mgm. $CaCO_3$, 50 mgm. K_2HPO_4, 25 mgm. $MgSO_4$ and 1 gm. of finely divided filter paper were added to each flask. Two flasks from each group received no nitrogen; two received 25 mgm. $NaNO_3$ and two received 50 mgm. $NaNO_3$. The amount of carbon dioxide evolved during the 14-day incubation period and the amounts of cellulose decomposed were determined. The results are given in table 11.

The results presented in table 11 help to establish further the fact that there is a definite correlation between the available nitrogen present in the soil and the amount of cellulose decomposed, whether this is measured by the

evolution of carbon dioxide or by the amount of residual cellulose. The addition of 25 mgm. of $NaNO_3$ resulted in a proportionally much greater increase in the amount of cellulose decomposed than the addition of 50 mgm. of $NaNO_3$. The addition of 25 mgm. $NaNO_3$, or about 4 mgm. of available nitrogen, resulted in an increase of the amount of cellulose decomposed in 5A by 180 mgm., in other words, for every milligram of available nitrogen, there was an increase of 45 mgm. of cellulose decomposed. This ratio is found to be 54 to 1 in the case of 7A. It was pointed out elsewhere, that pure cultures of fungi will decompose about 30 mgm. of cellulose for every

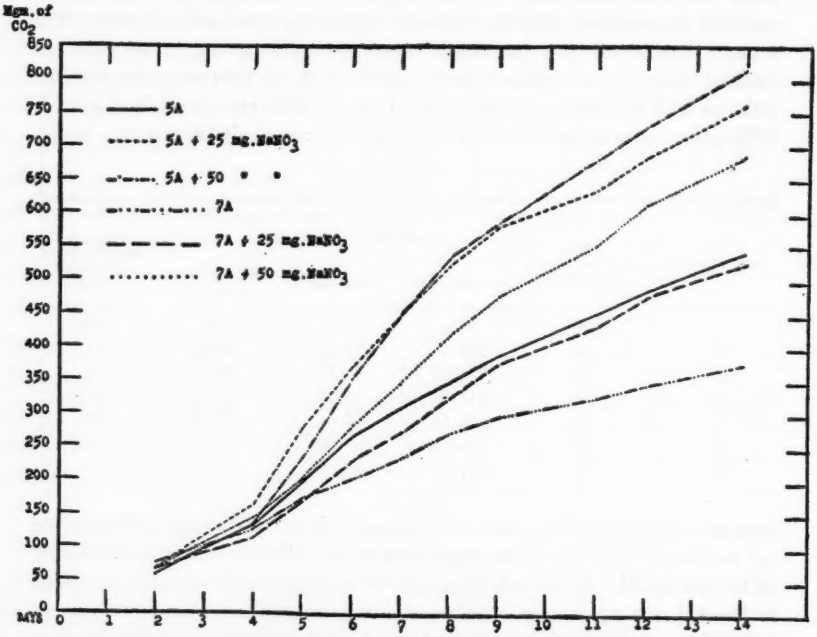

FIG. 1. INFLUENCE ON $NaNO_3$ UPON THE COURSE OF DECOMPOSITION OF CELLULOSE AS INDICATED BY THE EVOLUTION OF CARBON DIOXIDE

1 mgm. of nitrogen assimilated. In view of the fact that the bacteria require a much smaller amount of nitrogen for the synthesis of their protoplasm for the same amount of energy used up, the wider ratio between the amount of cellulose decomposed and nitrogen assimilated (45 to 54:1) is due to the activities of bacteria, in addition to the fungi; this is especially true, since these soils have been treated with calcium carbonate which has made the reaction favorable for the development of the bacteria. The influence of available nitrogen upon the course of cellulose decomposition, as indicated by the evolution of carbon dioxide is given in figure 1.

It is interesting to compare the influence of nitrogen upon cellulose decomposition in untreated soil and in soil to which a small amount of $CaCO_3$ (0.2 per cent), K_2HPO_4 (0.05 per cent) and $MgSO_4$ (0.025 per cent) have been added; this can be done by comparing the results in tables 4 and 11. When no lime and minerals are added to an infertile acid soil (7A—table 4), the addition of 25 mgm. of $NaNO_3$ brought about an increase of only about 5 per cent in the amount of cellulose decomposed, while the addition of 100 mgm. $NaNO_3$ resulted in an increase of 23 per cent cellulose decomposed; in the case of the fertile soil (5A—table 4), the addition of 25 mgm. $NaNO_3$ brought about an increase of 24.5 per cent in the amount of cellulose decomposed and 100 mgm. $NaNO_3$, of 55 per cent. This shows definitely that although nitrogen is an important factor, there is still something in 5A, which makes this soil far more active, from a microbiological view point. This may be due to the greater abundance of minerals and buffering substances, which make soil 5A decompose cellulose much more actively than 7A. When lime and minerals are added to these two soils (table 11) the addition of 25 mgm. $NaNO_3$ brought an increase of cellulose decomposition of 21.5 per cent in the unfertile soil (7A) and only 18 per cent in the fertile soil (5A); 50 mgm. $NaNO_3$ brought about an increase in the amount of cellulose decomposed by 33.5 per cent in 7A and by 29 per cent in 5A. In other words, when lime and minerals are added to two soils, one of which is very fertile and one very infertile, the addition of nitrogen stimulates cellulose decomposition about alike in both soils. But when no lime and minerals are added, the addition of available nitrogen will stimulate cellulose decomposition to a much greater extent in the fertile than in the infertile soil.

SUMMARY

The results brought out in this paper indicate that the determination of the power of a soil to decompose cellulose can be added to the group of methods used for carrying out a microbiological analysis of a soil. A study of the processes of nitrification brought out that the information obtained by this method can serve both as a basis for determining the microbiological condition of the soil, the rapidity with which the soil nitrogen becomes available, as well as the soil reaction and the buffer content of the soil. In a similar manner, the determination of the power of a soil to decompose cellulose can yield information on the microbiological condition of the soil as well as on its available nitrogen.

For every milligram of nitrogen that is available or that can become available in the soil, in the particular period of time, there will be decomposed approximately 40 to 50 mgm. of cellulose in the given amount of soil.

A study of the cellulose-decomposing power of the soil, both without and with the addition of a small amount of available nitrogen, can thus yield information for the differentiation of soil fertility, just as the determination

of numbers of microörganisms, of the nitrifying capacity and of the respiratory power of the soils. This method can supply information on the total available nitrogen in the soil as well as on the differences in the activities of the soil microörganisms. The results obtained by means of this method, when added to those obtained by the other methods, allow us to look forward towards having as complete a picture as possible of the microbiological processes in the soil and the chemical and physical condition of the soil, which affect these processes and which together go to make up soil fertility.

To measure the cellulose-decomposing power of the soil, three methods are recommended:

1. One gram of finely cut or well ground filter paper is well mixed with 100 gm. of fresh sieved soil; this is then placed in a tumbler, brought to optimum moisture, covered and incubated for 42 days, at 25–28°C, with frequent additions of water to keep the soil at optimum moisture. The amount of residual cellulose is determined in the soil, which is first air dried, by the method developed by Charpentier. The residual cellulose is then subtracted from the amount of cellulose originally present in the soil, determined by extracting 20 gm. of the original soil and 200 mgm. of the original paper, giving the amount of cellulose actually decomposed in the soil.

2. One gram of well ground filter paper and 100 mgm. of sodium nitrate are added to 100 gm. of soil, which is then well mixed and placed in a tumbler, brought to optimum moisture, covered and incubated for fifteen days. The amount of cellulose decomposed is determined in a manner similar to that used in the first method.

3. One hundred grams of soil, two hundred mgm. $CaCO_3$, 50 mgm. K_2HPO_4, and 25 mgm. $MgSO_4$, with and without 1 gm. of cut or ground filter paper, is placed in a respiratory apparatus and the amount of carbon-dioxide given off determined for fourteen days. The excess of carbon dioxide produced and the amount of cellulose decomposed in the soil containing cellulose, over the soil containing the minerals only, will serve as an index of cellulose decomposition and, *ipse facto*, of the available nitrogen in the soil. For every milligram of nitrogen that is available in the soil or that can become available in the given period of time, 40 to 50 mgm. of cellulose will be decomposed.

Every one of these three methods yields information on the cellulose-decomposing power of the soil viewed for a different angle. The first method, or the determination of cellulose decomposition in the untreated soil, supplies us with information as to the available nitrogen in the soil, when not treated further. The second method, or the determination of rapidity of cellulose decomposition in the soil, in the presence of available nitrogen, supplies information on the physical and chemical condition of the soil bearing upon microbiological activities and soil fertility, outside of the nitrogen factor: the addition of nitrogen will bring about greater decomposition of cellulose in the more fertile soil, with a more active microbiological flora. The third method, namely the determination of the cellulose decomposing capacity of the soil, in the presence of $CaCO_3$, K_2HPO_4 and $MgSO_4$, the lack of which may become limiting factors to cellulose decomposition, supplies information on the nitrogen of the soil which can become available, when the soil is properly limed and the necessary amount of mineral elements is added.

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SOME OBSERVATIONS ON THE DECOMPOSITION OF ORGANIC MATTER IN SOILS[1]

ROBERT L. STARKEY[2]

New Jersey Agricultural Experiment Stations

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INTRODUCTION

The carbon dioxide evolved from pure and mixed cultures of microörganisms in artificial and soil media has frequently been used to measure microbiological activity and decomposition of organic matter. Production of carbon dioxide, however, is seldom an absolute index of these processes.

When the decomposition of organic matter is measured by the amount of CO_2 produced, it should be kept in mind that the CO_2 is not the only product formed from the carbon of the organic matter in the process. The autotrophic microörganisms reassimilate some of the CO_2 while all heterotrophic organisms reassimilate some of the carbon during the process of growth and reproduction before it has completely decomposed; the fungi reassimilate much more carbon than the bacteria per unit of organic matter decomposed. Carbon dioxide is also not the only carbonaceous product of decomposition of organic matter even under aerobic soil conditions, since various intermediate products may be formed, while some of the constituents of the organic matter may be more resistant to decomposition than others. In general, however, the incomplete decomposition products of some organisms are further attacked by others and sooner or later appear as CO_2. The work of Bail (2), Neller (16) and Potter and Snyder (19) clearly show that cultures of bacteria, fungi and yeasts used in mixtures as well as the whole soil infusion produced much more CO_2 from sterilized organic matter than do any pure cultures of microörganisms.

Apparently the autotrophic microörganisms make up a small portion of the total numbers of microörganisms in the soil and it is undoubtedly true that, in the presence of an abundant and varied flora and under aerobic conditions, the organic matter undergoes relatively complete oxidation and only comparatively small amounts of incomplete oxidation products are

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formed and accumulate. The CO_2 produced from soils should, therefore, give a reliable although not an absolute index of the decomposition of organic matter. With this in mind, in considering later the amounts of organic materials decomposed, we shall be able to realize that the recorded results on CO_2 production are lower than the actual amounts of organic matter decomposed.

HISTORICAL

Source of soil organic matter. The organic matter in soils is largely replenished by the whole plants where they are not removed, roots and stubble from cut crops and organic manures added to the soil. Plant residues left in the soil may amount to 10–50 per cent of the organic matter removed in the cut crops [van Suchtelen (24)].

Relative ease of decomposition of certain materials and some factors involved. Wollny's extensive fundamental work (30) is still outstanding among the contributions on the decomposition of organic matter. Wollny found that the amounts of nitrogen contained in the organic materials greatly affect their rapidity of decomposition. Materials poor in nitrogen and rich in cellulose (cereal straws) decomposed more slowly than those rich in nitrogen but poor in cellulose (legume straws). Bone, guano, meat and bird excrements decomposed most quickly; the straws used for fertilizers and litters come next; then were listed the more common barnyard manures; hides, hoofs, and forest litters decomposed with difficulty; peat was the most resistant of all those studied.

Dvořák (4) stated that materials rich in oxygen and poor in carbon decomposed more quickly than those rich in carbon and poor in oxygen. He observed the following percentage decomposition of certain substances, as measured by the carbon dioxide produced in twenty-one days from 10 gm. of material added to 100 gm. of soil:

	per cent		*per cent*
Clover	59.7	Oak leaves	17.7
Glucose	42.1	Wheat straw	14.5
Rice straw	29.0	Cellulose	11.8
Levulose	27.2		

The rates of decomposition of these substances were likewise distinctive.

Fraps (5) reported that cottonseed meal, corn chops, rice hulls, and wheat shorts decomposed more rapidly (72–81 per cent in 14 weeks) than meat, blood and tankage (47–68 per cent in 14 weeks) as measured by loss upon ignition. These results are quite contrary to those observed by Wollny. As measured by production of carbon dioxide, the decomposition of wheat bran was greatest, followed by cottonseed meal, corn chops, manure, and cobs. Waksman (25) states that in the presence of large quantities of carbohydrates, molds compete with higher plants for available nitrogen compounds. Upon decomposition of the mold mycelium, the nitrogen is given back to the soil. Potter and Snyder (19) further showed that sterilized soils, with and without dextrose, inoculated with fungi decreased in nitrate nitrogen. Green manure decomposed much more rapidly than barnyard manure (18). In fifty-three days clover was 60.8 per cent decomposed, oats 49 per cent, and manure 42 per cent. Manure is naturally composed of decomposed organic residues which would decompose further only slowly. In work similar to some previously conducted by Wollny, Merkle (13) noted legumes decomposed more rapidly than straw and litters low in nitrogen. Sugar beets decomposed rapidly for a time. Swedes and rape gave nearly as much carbon dioxide as legumes. Forest litter gave less carbon dioxide than non-legumes and root crops. No very great differences in the rates of decomposition of these substances were noted contrary to many results of others.

The subject of nitrogen starvation of plants as a result of application of straw has received much attention. Kellerman (10) noted injury to citrus trees due to nitrogen starvation after fertilization with mature barley straw.

Rahn (20) studied the effects of the presence of available nitrogen on straw decomposition as measured by the loss of weight upon ignition and carbon dioxide produced. He found that nitrate or ammonium nitrogen accelerated the decomposition of the straw two days after its application to the soil; the nitrate appeared to be superior. The straw treated soils normally became deficient in nitrogen, but legumes did not cause this effect. As soon as the most of the organic matter has decomposed, there follows a slow and steady formation of ammonia and nitrate which is greater than the amounts originally present. There is apparently little or no loss of nitrogen by denitrification. After fifteen days, in the presence of nitrate, 15 per cent of the straw was decomposed. Manure, or straw composted with liquid manure containing more nitrogen than straw alone, did not cause nitrogen deficiencies in soils. Murray (15) studied the straw problem further and found that the excess energy material low in nitrogen greatly increased the numbers of bacteria temporarily, which organisms assimilated a proportionate amount of soluble nitrogen and other plant food elements. The straw did not, however, increase any one group of bacteria more than the others. He observed no loss in total nitrogen in the soil nor inhibition to the nitrification or ammonification processes. Scott (22) and Stephenson (23) noted similar effects from straw fertilization.

Lyon, Bizzell and Wilson (11) observed that roots of oats, timothy, and corn, upon decomposition, depleted the soil's store of nitrate far below that of the untreated soil. Clover had hardly any effect while dried blood increased the soil nitrates. These effects corresponded with the nitrogen contents of the materials. Gainey (6) noted effects similar to those from straw fertilization following treatment of the soil with paraffin oil.

Miyake and Nakamura (14) made use of the production of carbon dioxide in following the decomposition of soybean and herring cakes. They found that CaO stimulated decomposition more than did $CaCO_3$, but these sources of lime increased the soluble nitrogen in the reverse order, the amounts of organic nitrogen mineralized not being proportional to the amounts of organic matter decomposed. It is quite probable that the effects of lime on the decomposition of organic matter rich in nitrogen such as they used (7–11 per cent nitrogen) would be different from those on organic matters lower in nitrogen.

Waksman and Starkey (26) noted the decomposition of 43 per cent of alfalfa meal two weeks after its addition to soil. Potter and Snyder (17) recorded that increased amounts of carbon dioxide were produced from soils receiving increasing applications of manure. Lime further increased this carbon dioxide. Wollny (30) clearly demonstrated that carbon dioxide produced from soils to which different amounts of organic matter were added was not proportional to the amounts of these additions, due to the deficiency of oxygen and the sterilizing action of large amounts of carbon dioxide.

Influences of the nature of the organic matter upon the rapidity of its decomposition. Wollny (30) states that the intensity of the decomposition of organic matter largely depends upon the quantity and quality of the material added and its state of division. Organic matter which usually decomposes only with difficulty, as in the case of peat, can be made to decompose more easily if finely divided; in the case of easily decomposable material, such as pea vines, the fineness is relatively unimportant. Wollny and also Whiting and Schoonover (29) noted that plants in the green state decompose more readily than after drying and subsequent remoistening. These last named authors believed that curing hindered decomposition both by altering the chemical composition of the material and by destroying enzymes which facilitated decomposition of the green material by autolysis. The material was believed to become more horny and shriveled by drying. The leaves of plants decomposed more rapidly than their stems or roots; this was correlated with the higher nitrogen content of the leaves. This has also been shown more recently by Bal (3) and Martin (12) who found that younger and more succulent plants decomposed more rapidly. Dvořák (4) states that the fresh plant residues are most available largely since the lower carbohydrates are more abundant; in the older plants, the ligno-celluloses predominate.

Rates of decomposition. It has been repeatedly noticed that the decomposition of the more readily decomposable organic materials was greatest soon after their addition. The time

varied with the kind of organic matter used and the environmental conditions. Wollny states that organic matter decomposes with more difficulty as the alteration or decomposition is advanced. Van Suchtelen (24) mentions that, in general, the less abundant hexoses and pentosans decompose first followed by the polysaccharides, celluloses, pectins, starches, and albumins. A strongly resistant carbonaceous residue is left which, however, is decomposed slowly. Dextrose gave the maximum production of carbon dioxide the second day while green manure (oat straw) gave the maximum the sixth day (temperature used 10–12°C.). Fraps (5) found that oxidation was very rapid at the start and decreased after the first week. Potter and Snyder (18) found that clover decomposed rapidly while oats had a slow initial rate of decomposition. The carbon dioxide produced from inoculated sterile soils (19) was greatest the first few days whether additional organic matter was added or not. Gainey (7) obtained the maximum carbon dioxide the second day from dried blood and cottonseed meal, the former decomposing more rapidly. Hibbard (9), Rahn (20), Dvořák (4) and Waksman and Starkey (26) noted quite similar effects the first few days after the addition of organic matter to soils.

Decomposition by soils of different fertility. Wollny records that decomposition is more active in cultivated than in rarely cultivated soils. Fraps (5) states that the relative powers of soils to oxidize added organic matter are not very variable, but that the relative rates of production of carbon dioxide from the soils themselves show greater variation. Quite similar results have been repeatedly obtained in this laboratory

Correlations have been made between the decomposing powers of different soils and their fertility. This subject is somewhat completely discussed elsewhere (27).

Effects of organic matter on numbers of microörganisms. Since carbon dioxide is produced from the organic matter by microörganisms there occurs a simultaneous increase in the numbers of these organisms with the increase in carbon dioxide. Bacteria, fungi and actinomycetes all are prominent in organic matter decomposition, not all, however, being affected alike by different organic materials. This question has been recently considered in detail by Waksman and Starkey (28). Van Suchtelen concluded that production of carbon dioxide was a much more accurate index of the numbers and activities of these organisms than the numbers determined by the present plate and dilution methods. Russell (21) makes the following qualitative explanation of the effects of organic materials on microbiological activities:

	CHANGES IN NO_3 AND NH_3	NUMBERS OF MICROÖRGANISMS
Soil under normal conditions	Accumulation	Low and fluctuating
Soil plus carbohydrates	Decrease	Rapid increase then a decrease
Soil plus peptone	Increase	Rapid increase and rapid decrease

Injurious effects of some organic materials. Some of the organic matters added to, or occurring in soils have an injurious effect upon decomposition. Wollny states that the presence of resins, waxes, fats, and tannins, frequently occurring in peats are partly responsible for their slow decomposition. Dvořák believed that the terpenes and tannic acid in some organic materials had an unfavorable action on bacterial activity. Gibbs and Werkman (8) found sawdust, leaves, needles, cones and various tree products inhibited nitrification and ammonification and several years might pass before the detrimental materials disappeared.

METHODS

The soils used were put through a 3-mm. sieve after being brought to the laboratory. The alfalfa meal and rye straw were obtained a few weeks be-

fore starting the experiments, dried, and ground to pass through a ½-mm. sieve. The cellulose was used in the form of finely cut filter paper. In general, one gram of the organic matter studied was incorporated with 100 gm. of soil. Distilled water was added in such amounts as to bring the soil to optimum moisture content. In some instances one gram of organic matter was used in 200 gm. of soil. Where dextrose was used, 500 mgm. were added in solution to 100 gm. of soil. The soils were kept in a constant temperature room at 25–28°C. as was also the apparatus used for determining the carbon dioxide produced from the soils. The apparatus used has been described in detail elsewhere (16, 27). Both the large and the small apparatus used in previous experiments has been used in these studies. Air free from carbon dioxide was constantly circulated over the surface of the soils at the rate of three liters per hour. Measurement of the carbon dioxide produced from these organic materials has given very satisfactory results. In all cases the treatments were run in duplicate which determinations showed close agreements for work of this kind.

Total carbon determinations were made according to the official method of the Association of Agricultural Chemists (1). The nitrate determinations were made by the phenoldisulfonic acid method. Ammonia was determined by magnesium oxide distillation.

RESULTS

Decomposition of different organic materials in the same soil. In these experiments organic materials of widely different character have been used. Alfalfa meal and rye straw have been taken as examples of two types of organic matter commonly added to soils as green manures. Dried blood and cellulose have been used as examples of organic materials rich and poor in nitrogen, one of animal origin and readily decomposed, the other of plant origin and generally considered quite resistant to decomposition. The fungous material that has been used was obtained as mycelial and spore growth from cultural media, washed, dried and finally ground.[3] The question has frequently been raised as to the availability of the nitrogen which becomes stored in fungous and bacterial bodies. It seems that the carbon dioxide produced from such material should answer this question in part.

The amounts of nitrogen and carbon contained in the materials used are listed below:

	NITROGEN	CARBON		NITROGEN	CARBON
	per cent	*per cent*		*per cent*	*per cent*
Cellulose	0.00	44.44	Fungous material	3.84	44.31
Rye straw	0.72	36.40	Dried blood	9.61	37.52
Alfalfa meal	2.45	40.62	Dextrose	0.00	37.46

[3] The author is obliged to Mr. Heukelekian of this laboratory for the fungous material.

The air stream, after passing over the soils containing dried blood and fungous material, passed through standard sulfuric acid to remove the ammonia. All the organic materials except the cellulose were added to a fertile loam soil in amounts equivalent to one-half per cent of the weight of the soil. One per cent of cellulose was added. The data for this experiment are all calculated on the basis of 0.5 gm. of organic matter. Table 1 and figure 1 present the results. In this figure and those following, in place of plotting the actual amounts of carbon dioxide produced, the percentage of the carbon contained in the organic matter which was given off as carbon dioxide at the different intervals has been plotted. In all instances, the carbon dioxide from soils similarly treated but lacking the organic additions

TABLE 1

Rates (I) and Courses (II) of decomposition of some organic materials

ORGANIC MATTER USED	AFTER DIFFERENT PERIODS OF INCUBATION (DAYS)										TOTALS
	1	2	3	4	5	6	7	8	9	10	
I. Daily production of CO_2 in mgm.											
Cellulose	1.83	1.84	1.83	7.14	6.87	28.05	28.04	27.00	27.00	*33.46*	163.06
Rye straw	*74.29*	29.31	37.83	22.98	16.65	12.59	13.08	12.93	9.77	9.77	239.20
Alfalfa meal	*106.63*	67.83	68.24	37.42	27.79	15.48	14.58	13.76	8.33	8.32	368.38
Fungous material	*79.80*	44.44	63.70	62.46	57.92	41.34	33.44	27.80	16.58	16.58	444.06
Dried blood	7.23	41.49	68.30	*82.33*	82.32	56.72	48.61	31.40	26.05	23.15	467.60
Dextrose	84.98	*156.52*									
II. Percentage decomposed at daily intervals											
Cellulose	0.23	0.45	0.68	1.55	2.39	5.84	9.28	12.59	15.90	*20.01*	
Rye straw	*11.13*	15.52	21.19	24.64	27.13	29.02	30.98	32.91	34.38	35.84	
Alfalfa meal	*15.98*	26.14	36.37	41.98	46.14	48.46	50.64	52.71	53.95	55.20	
Fungous material	*11.96*	18.62	28.16	38.20	46.20	52.40	57.41	61.57	64.06	66.54	
Dried blood	1.05	7.08	17.01	*28.98*	40.95	49.19	56.26	60.83	64.61	67.98	
Dextrose	12.37	*35.16*									

has been substracted from the presented data. It is accordingly assumed that this carbon dioxide has all been produced from the organic matter added.

It is naturally apparent that in the same soil the amount of decomposition of different organic materials at any one time is different. The first few days show the greatest divergence in decomposition. At the two days' interval they vary from less than 0.5 per cent of the cellulose to over 35 per cent of the dextrose decomposed. This wide variation holds to a large extent throughout the period. After ten days, 20 per cent of the cellulose, 35 per cent of the rye straw and considerably over 50 per cent of the alfalfa meal, fungous material and dried blood have become decomposed. Unfortunately the dextrose determinations were discontinued after two days.

Decomposition of these organic materials in soil is extremely rapid. Although the conditions used approach the optimum and are far more favor-

able than those in the field, it is only reasonable to consider that such organic materials would largely disappear even under normal field conditions in a very short time. The periods of maximum decomposition are indicated in the table by italics. It is noted that, in general, the period of maximum decomposition occurs the first day after addition of these materials to the

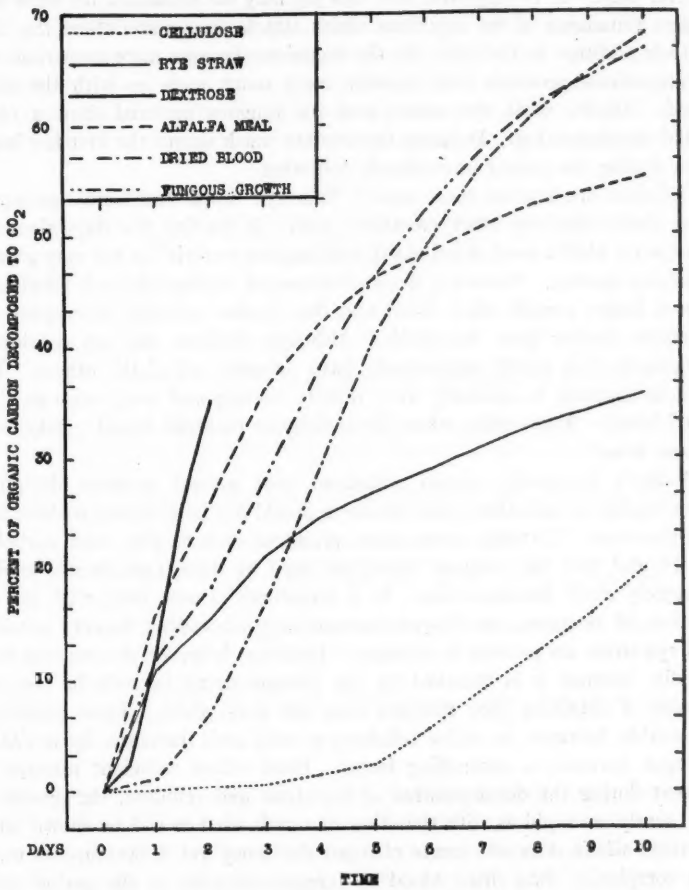

FIG. 1. COURSES OF DECOMPOSITION OF SOME ORGANIC MATERIALS IN SOIL

soil. This is the case with the rye straw, alfalfa meal, and fungous material. Dextrose shows the maximum decomposition the second day. Although this material was not studied further here, numerous experiments have indicated the assumption to be correct that dextrose decomposes most rapidly the first 48 hours in fertile soil. In the case of cellulose the decomposition

is most rapid on the tenth day. The dried blood gave maximum daily decomposition the fourth day.

The rate of decomposition with each organic material is distinctive. Cellulose exhibits a very marked and extended lag before the decomposition becomes at all rapid. Dried blood also exhibits this lag although for a much shorter time. It is suggested that this lag may be accounted for by a lack of any abundance of the organisms which attack these materials at the time of their addition to the soil. As the organisms become more numerous, the decomposition proceeds more rapidly, very much more so with the dried blood. Alfalfa meal, rye straw, and the fungous material show a rapid initial decomposition. Dextrose decomposes much slower the first few hours than during the period immediately following.

Cellulose decomposes more slowly than rye straw and both decompose more slowly than the other substances used. If the first few days alone are considered, alfalfa meal, dried blood, and fungous material do not vary greatly from one another. However, if completeness of decomposition is considered over a longer period, dried blood and the fungous material decompose appreciably further than the alfalfa. Although dextrose was not considered sufficiently, this would undoubtedly have exceeded all of the others. The fungous material is evidently very rapidly decomposed even more so than dried blood. Apparently, when dead, fungous material would quickly disappear in soil.

Wollny's frequently quoted statement that animal products disappear more rapidly in soils than plant products should not be accepted without any qualifications. Certainly some plant products such as the lower carbohydrates and also the fungous mycelium used in these experiments exhibit extremely rapid decomposition. It is important to note that, with the exception of dextrose, the slowest decomposing substances, namely cellulose and rye straw are poorest in nitrogen. Dextrose, however, decomposes very rapidly because it is attacked by the nitrogen-fixing bacteria in the soil, capable of obtaining their nitrogen from the atmosphere. These organisms are unable, however, to utilize celluloses as such and, therefore, the available nitrogen becomes a controlling factor. Even where sufficient nitrogen is present during the decomposition of rye straw and cellulose, the process is not nearly as rapid as with the other materials used as will be shown later. Further, alfalfa does not create nitrogen deficiency yet it decomposes much less completely than dried blood or fungous material in the period used. There are undoubtedly limitations to decomposition other than nitrogen deficiency.

Nitrogen exchanges in the presence of decomposing organic matter. The effects of nitrates on the decomposition of rye straw and alfalfa meal have been studied. One-gram portions of rye straw and alfalfa meal were added to 200-gm. portions of soil with and without 0.05 gm. $NaNO_3$. The results are given in table 2 and figure 2. Nitrate has not increased the decomposi-

tion of alfalfa meal, in fact the soil to which nitrate has been added gave less carbon dioxide than the one lacking it. The differences between the two are not believed to have been caused by the nitrate. Nitrate distinctly favored straw decomposition. This effect of the nitrate was noticed on the third day and continued to increase decomposition over the straw lacking the nitrate to the end of the period.

The nitrogen exchanges in the presence of rye straw, alfalfa meal, and dried blood may throw light upon the decomposition of these organic materials. In table 3 are listed the nitrate and ammonia determinations from soils treated with these organic materials with and without nitrate. As would be expected, the ammonium and nitrate nitrogen increased in the untreated soil.

TABLE 2

Decomposition of rye straw and alfalfa meal with and without added nitrates

TREATMENT	PERIOD OF INCUBATION (DAYS)										TOTALS
	1	2	3	4	5	6	7	8	9	10	
Daily production of CO_2 in mgm.											
Rye straw[1]	48.33	*52.96*	42.55	34.14	29.23	27.20	22.57	24.02	19.68	22.57	323.25
Rye straw + $NaNO_3$	43.12	53.83	*69.16*	68.29	57.88	55.85	46.88	47.46	37.91	39.06	519.44
Alfalfa meal	170.84	*174.51*	109.40	70.32	50.06	41.67	30.96	29.81	22.57	24.31	724.45
Alfalfa meal + $NaNO_3$	*180.29*	156.56	102.73	63.95	44.85	38.49	27.78	25.18	19.68	21.12	680.63
Percentage decomposition at daily intervals											
Rye straw	3.13	*6.55*	9.30	11.51	13.40	15.16	16.62	18.17	19.45	20.91	
Rye straw + $NaNO_3$	2.79	6.27	*10.74*	15.16	18.90	22.52	25.55	28.62	31.07	33.59	
Alfalfa meal	11.71	*23.66*	31.16	35.98	39.41	42.27	44.39	46.43	47.98	49.85	
Alfalfa meal + $NaNO_3$	*13.36*	23.08	30.12	34.50	37.58	40.21	42.12	43.84	45.19	46.64	

[1] Rye straw = 42.17 per cent C.

In the presence of rye straw the nitrate nitrogen entirely disappeared and the ammonium nitrogen did not change appreciably from the control. At the end of the incubation period this soil had a deficiency in soluble nitrogen over that in the control. Where the nitrate was added with the straw the ammonium nitrogen remained unchanged, but the nitrate increased almost reaching that in the control. With the alfalfa meal alone the ammonia did not change but the nitrates increased showing a marked increase in soluble nitrate over the control. There was also an increase in nitrate nitrogen where nitrate was added with the alfalfa, this being twice as great as in the absence of added nitrate. Where the dried blood was added, both the ammonium and nitrate nitrogen increased, the first more than seven times as much as

the second, resulting in a remarkably high increase in soluble nitrogen. It is observed that ammonia was brought over in the air current only from the soils treated with dried blood, in which case the decomposition liberated more ammonia than the soil could hold.

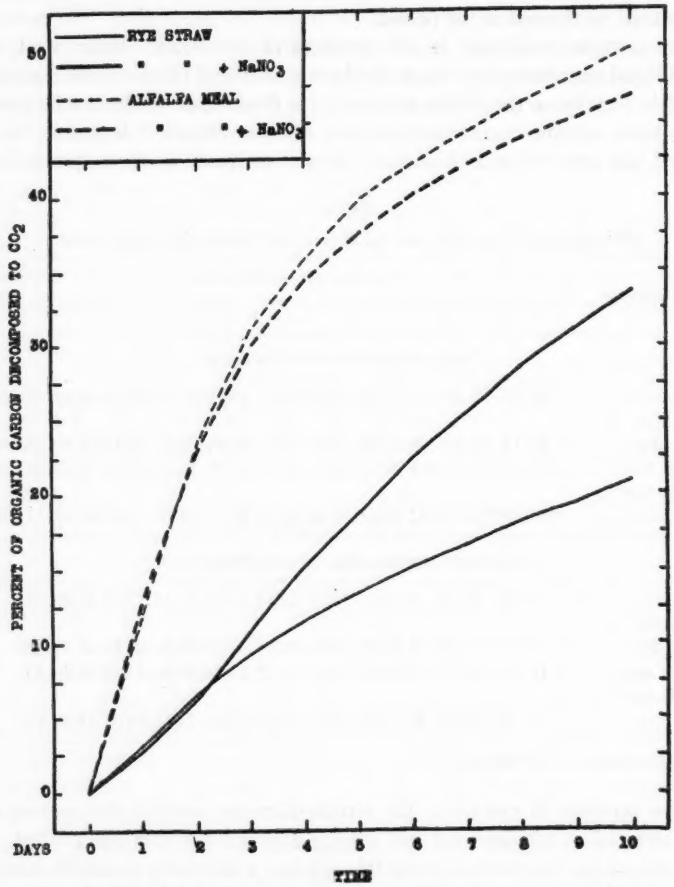

FIG. 2. EFFECTS OF NITRATE ON THE COURSES OF DECOMPOSITION OF RYE STRAW AND ALFALFA MEAL

Apparently there was no nitrogen deficiency except where straw was used without additional nitrate. The amounts of organic matter decomposed, as determined by the carbon dioxide produced should free a definite amount of nitrogen to be either reassimilated or given off as a waste product in the form of ammonia which becomes oxidized more slowly to nitrate. The last

column in table 3 gives this calculated amount of nitrogen. In the case of rye straw and the alfalfa without nitrate there is a considerable deficiency in soluble nitrogen accumulated as compared with the amount probably liberated. However, where nitrate was added with the alfalfa meal and with dried blood there is an excess accumulation over that assumed to be liberated.

TABLE 3

Ammonium and nitrate nitrogen transformations in soils treated with some organic materials

SOIL TREATMENT	NITROGEN CONTENT AT START			NITROGEN CONTENT AT END					INCREASE OR DECREASE IN SOLUBLE N	INCREASE OR DECREASE IN N OVER CONTROL	N ADDED IN ORGANIC MATTER	CALCULATED N IN ORGANIC MATTER DECOMPOSED
	NH_3-N	NO_3-N	Total soluble N	NH_3-N in soil	NH_3-N in air stream	Total NH_3-N	NO_3-N	Total soluble N				
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Untreated....	1.74	0.16		2.04	0.36		2.13					
Untreated....				2.60	0.07		5.11					
Average.....	*1.74*	*0.16*	*1.90*	*2.32*	*0.22*	*2.54*	*3.62*	*6.16*	*4.26*			
Rye straw....	1.62	0.12		3.16	0.07		0.00				7.14	
Rye straw....				2.04	0.22		0.00				7.22	
Average.....	*1.62*	*0.12*	*1.74*	*2.60*	*0.15*	*2.75*	*0.00*	*2.75*	*1.01*	*−3.25*	*7.18*	*1.78*
Rye straw + NO_3.....	1.74	3.09		1.44	0.36		6.71					
Rye straw + NO_3.....				1.44	0.45		7.24					
Average.....	*1.74*	*3.09*	*4.83*	*1.44*	*0.40*	*1.84*	*6.98*	*8.82*	*3.99*	*−0.27*	*7.18*	*2.94*
Alfalfa meal..	2.46	0.20		2.04	0.36		11.71				24.75	
Alfalfa meal..				2.60	0.00		9.94				24.25	
Average.....	*2.46*	*0.20*	*2.66*	*2.32*	*0.18*	*2.50*	*10.83*	*13.33*	*10.67*	*6.41*	*24.50*	*13.36*
Alfalfa meal + NO_3.....	1.74	4.39		2.04	0.00		23.95					
Alfalfa meal + NO_3.....				2.60	0.00		20.76					
Average.....	*1.74*	*4.39*	*6.13*	*2.32*	*0.00*	*2.32*	*22.36*	*24.68*	*18.55*	*14.29*	*24.50*	*12.46*
Dried blood...	1.62	3.19		58.00	7.00		16.93				86.45	
Dried blood...				75.92	14.94		10.64				105.79	
Average.....	*1.62*	*3.19*	*4.81*	*66.96*	*10.97*	*77.93*	*13.79*	*91.72*	*86.91*	*82.65*	*96.12*	*73.00*

The organic materials added were composed of many compounds and apparently either the parts higher in nitrogen were decomposed in these instances or else the organic matter decomposed considerably further than the carbon dioxide produced indicated, both of which would tend to explain these differences.

It may be concluded from these results on the early stages of organic matter decomposition (1) that rye straw tends to deplete soluble soil nitrogen, (2) that alfalfa does not greatly affect the supply of soluble soil nitrogen, not drawing on this supply but slightly adding to it, and (3) that dried blood immediately increases the soil's supply of soluble nitrogen considerably, the ammonia in particular, which more slowly becomes oxidized. These effects closely correlate with the percentages of nitrogen in the organic materials (see pages 10 and 11).

Decomposition in soils of different fertility. The soils used were from the experimental plots and varied greatly in fertility:

SOIL NUMBER	TREATMENT	FERTILITY
5A	Manure	Very fertile
5B	Manure plus lime	Very fertile
7A	Untreated	Extremely poor
7B	Lime	Fair
9A	$NaNO_3$ plus K and P minerals	Good
11B	$(NH_4)_2SO_4$ plus K and P minerals plus lime	Good

In these studies 1-gram portions of organic matter in the forms of cellulose, rye straw and dried blood were added to 100-gm. portions of soil. Additions of 0.10 gm. $NaNO_3$ were made to some of the soils containing cellulose. The incubation period used for cellulose was longer than usual since in a short time there was very little decomposition. As previously, in the case of the dried blood, the air stream passed through standard sulfuric acid after being drawn over the soil in order to trap the ammonia given off. Dextrose has been used in similar experiments reported elsewhere (27) and this organic matter is given consideration at this point. The results are found in tables 4–6 and figures 3–5.

The most evident fact is that decomposition of the same material is different in different soils. However, the order of the rapidity with which the different soils decompose one organic matter is not the same for a different organic material. It has previously been reported concerning decomposition of dextrose and alfalfa (27) that they effect greater evolution of carbon dioxide from soils that had received lime treatment than from similar soils not treated with lime. In general, the carbon dioxide produced was also correlated with the amounts of crops produced from these soils. The production of carbon dioxide from dextrose in soils varies during the first few days more than from most of the other organic materials studied.

The decomposition of cellulose, rye straw and dried blood shows somewhat similar relationships although this is not at all consistent. Cellulose in particular shows this. Further, the acceleration of decomposition of cellulose caused by nitrate is also very apparent here as shown previously with rye straw. Even under particularly favorable conditions in the fertile soil 5A,

TABLE 4

Decomposition of cellulose in soils of different fertility

TREATMENT	PERIOD OF INCUBATION (DAYS)																	TOTALS
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Daily production of CO_2 in mgm.																		
Soil 5A	4.37	4.37	4.37	12.90	12.37	*18.14*	18.14	17.14	17.14	14.84	14.84	14.84	10.54	10.54	11.73	11.73	11.73	209.73
Soil 5A + $NaNO_3$	3.67	3.67	3.67	14.27	13.74	56.09	56.09	53.99	53.99	*66.91*	66.91	66.91	41.06	41.06	32.63	32.63	32.63	639.92
Soil 7A	1.25	1.25	1.25	0.33	0.33	2.88	2.88	2.54	2.54	4.27	4.27	4.27	*4.46*	4.46	4.46	4.46	4.46	50.36
Soil 7A + $NaNO_3$	0.15	0.15	0.15	0.61	0.61	7.27	7.27	6.93	6.93	13.26	13.26	13.26	13.26	13.26	*17.48*	17.48	17.48	148.81
Soil 7B	3.91	3.91	3.91	3.91	5.36	5.09	5.09	*7.27*	7.27	5.25	5.25	5.25	4.63	4.63	4.63	4.63	4.63	84.62
Soil 7B + $NaNO_3$	2.44	2.44	2.44	6.11	7.56	20.49	20.49	*33.95*	33.95	30.55	30.55	30.55	33.12	33.12	27.99	27.99	27.99	371.73
Percentage decomposed at daily intervals																		
Soil 5A	0.27	0.54	0.80	1.60	2.36	*3.47*	4.58	5.63	6.69	7.60	8.51	9.42	10.06	10.71	11.43	12.15	12.87	
Soil 5A + $NaNO_3$	0.23	0.45	0.68	1.55	2.39	5.84	9.28	12.59	15.90	*20.01*	24.06	28.22	30.74	33.26	35.26	37.27	39.27	
Soil 7A	0.08	0.15	0.23	0.25	0.27	0.45	0.62	0.78	0.94	1.20	1.46	1.72	*2.00*	2.27	2.54	2.82	3.09	
Soil 7A + $NaNO_3$	0.01	0.02	0.03	0.07	0.10	0.55	0.99	1.42	1.85	2.66	3.47	4.29	5.10	5.91	*6.99*	8.06	9.13	
Soil 7B	0.24	0.48	0.72	0.96	1.29	1.60	1.91	*2.36*	2.81	3.13	3.45	3.77	4.06	4.34	4.62	4.91	5.19	
Soil 7B + $NaNO_3$	0.15	0.30	0.45	0.82	1.29	2.55	3.80	*5.89*	7.97	9.84	11.72	13.28	15.63	17.66	19.38	21.09	22.81	

TABLE 5

Decomposition of rye straw in soils of different fertility

SOIL	PERIOD OF INCUBATION (DAYS)										TOTALS
	1	2	3	4	5	6	7	8	9	10	
	Daily production of CO_2 in mgm.										
5A	*51.10*	45.08	20.13	20.12	14.86	14.86	14.86	11.59	11.59	11.58	215.77
5B	*56.00*	27.59	14.88	14.88	14.06	14.06	14.06	11.56	11.56	11.56	190.21
7A	*35.64*	19.93	12.46	12.46	10.10	10.10	10.10	6.74	6.74	6.74	131.01
7B	*49.88*	26.18	16.75	16.75	16.27	16.27	16.27	10.36	10.36	10.36	189.45
9A	32.34	*45.97*	19.11	19.11	16.97	16.97	16.97	9.01	9.01	9.01	194.47
11B	*53.31*	28.17	18.73	18.73	15.59	15.59	15.59	10.16	10.16	10.16	196.19
	Percentage decomposed at daily intervals										
5A	*3.83*	7.21	8.72	10.22	11.34	12.45	13.56	14.43	15.30	16.17	
5B	*4.20*	6.27	7.38	8.49	9.55	10.60	11.65	12.52	13.39	14.25	
7A	*2.67*	4.16	5.10	6.03	6.79	7.54	8.30	8.81	9.31	9.82	
7B	*3.74*	5.70	6.95	8.21	9.43	10.65	11.87	12.64	13.42	14.20	
9A	2.42	*5.87*	7.30	8.73	10.00	11.27	12.54	13.22	13.90	14.57	
11B	*3.99*	6.11	7.49	8.91	10.08	11.25	12.42	13.18	13.94	14.70	

TABLE 6

Decomposition of dried blood in soils of different fertility

SOIL	PERIOD OF INCUBATION (DAYS)										TOTALS
	1	2	3	4	5	6	7	8	9	10	
	Daily production of CO_2 in mgm.										
5A	19.00	73.35	104.00	97.97	90.63	*112.26*	90.43	103.44	69.06	65.30	825.44
5B	29.60	133.86	*165.81*	152.97	110.55	121.74	96.79	77.14	79.74	61.55	1029.75
7A	10.74	37.20	53.97	77.02	91.15	134.07	130.02	*143.31*	102.28	82.93	862.69
7B	29.08	71.52	91.95	111.34	98.23	*126.33*	88.55	83.21	79.17	60.39	839.77
9A	13.23	45.46	67.85	74.13	71.77	102.27	101.12	*103.15*	103.15	82.64	764.77
11B	35.24	86.05	110.94	*116.04*	94.92	112.52	79.45	73.68	58.08	41.61	808.53
	Percentage decomposed at daily intervals										
5A	1.06	6.07	13.31	20.11	26.33	*34.13*	40.35	47.57	52.30	56.75	
5B	1.87	11.32	*23.10*	33.93	41.67	50.22	56.96	62.32	67.86	72.08	
7A	0.72	3.37	7.24	12.78	19.32	28.31	38.33	*48.63*	55.95	61.86	
7B	1.80	6.68	13.05	20.83	27.76	*36.73*	42.96	48.77	54.30	58.46	
9A	0.86	4.06	8.88	14.16	19.27	26.59	33.79	*41.20*	48.58	54.46	
11B	2.32	8.34	16.17	*24.36*	31.07	39.05	44.63	49.80	53.84	56.69	

further improved by the addition of $NaNO_3$, the cellulose was only about 40 per cent decomposed in seventeen days as compared with dextrose which was more than 35 per cent decomposed in two days. The extremely slow initial decomposition of cellulose is strikingly apparent.

There are no very wide differences between the different soils with respect to their abilities to decompose rye straw. The production of carbon dioxide is generally uniformly low. In general, the richer soils tend to decompose rye straw more rapidly than the poorer soils. There were undoubtedly deficiencies in soluble nitrogen in all of these soils early in the stage of decomposition which limited the process later.

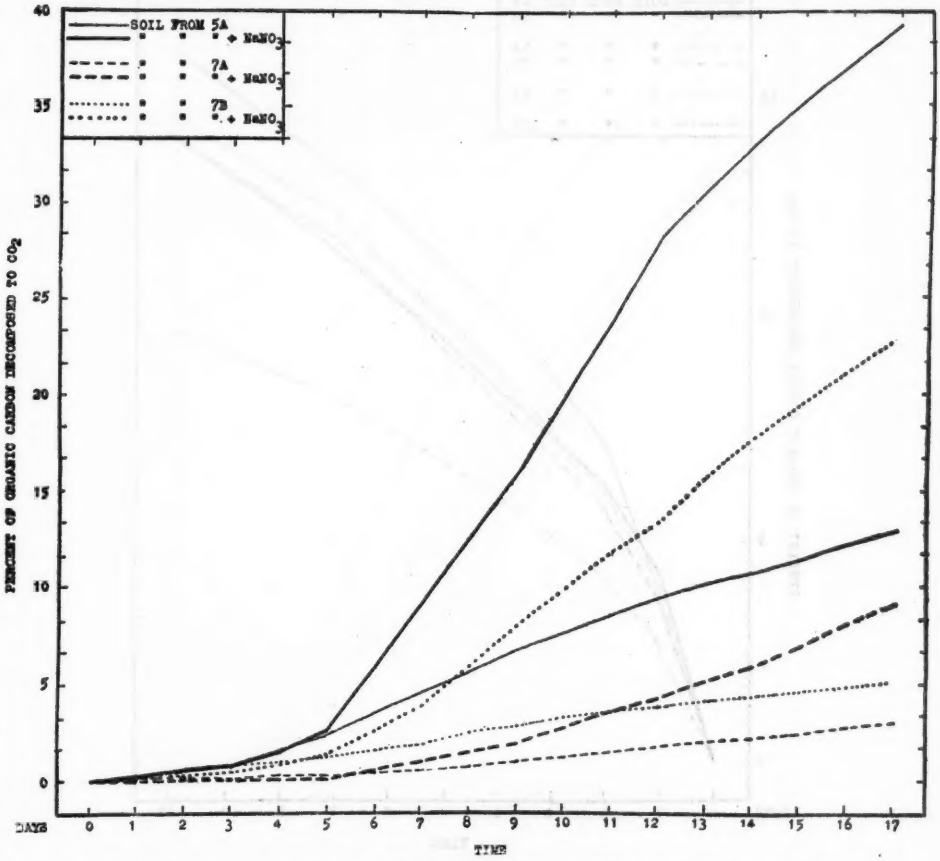

FIG. 3. EFFECTS OF NITRATE ON THE COURSES OF DECOMPOSITION OF CELLULOSE IN SOILS OF DIFFERENT FERTILITY

Decomposition of dried blood was very rapid in all of the soils following the characteristic initial lag. However, there is no consistent correlation here between the amount decomposed in the soils and their fertility. This is principally caused by the abundant ammonia production which tended to bring all of the soils to an alkaline condition. In the more nearly neutral

poorly buffered soils the reaction evidently became too alkaline for favorable decomposition.

The changes in soluble nitrogen in these soils are presented in table 7. The nitrate changes have been negligible, but the increases in ammonium nitrogen are considerable and account for practically all of the increase in

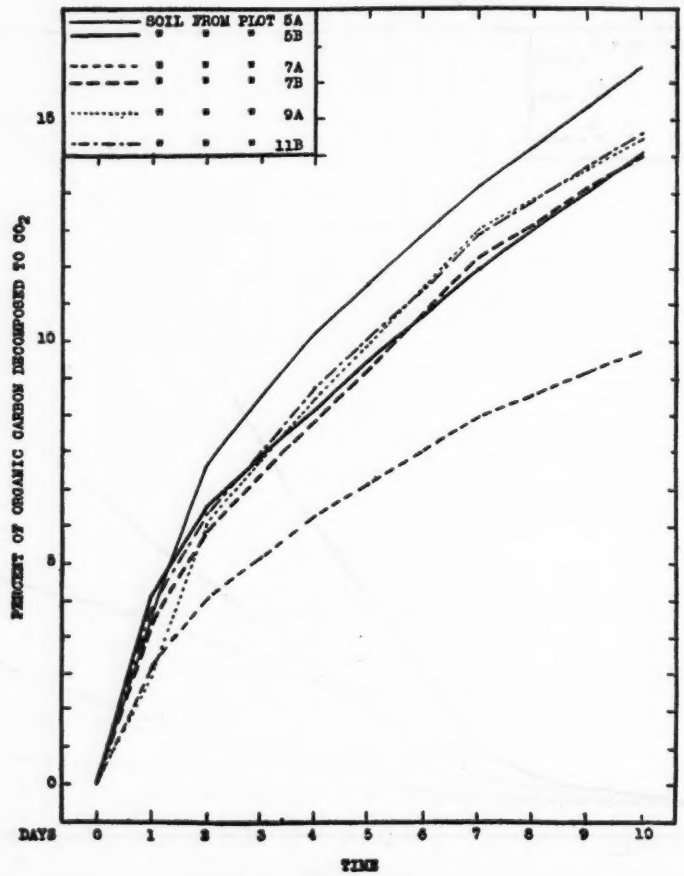

FIG. 4. COURSES OF DECOMPOSITION OF RYE STRAW IN SOILS OF DIFFERENT FERTILITY

soluble nitrogen in the soil, amounting to 35–70 mgm. in the different soils. A large part of the ammonia produced in the more nearly neutral soils was trapped from the air stream, while less was drawn from the more acid soils, more being retained in the soils themselves. A comparison between the determined increase in soluble nitrogen with that calculated to have been

liberated from the amounts of dried blood decomposed bring out some differences. It is noted that from the more acid soils (5A, pH 5.5; 7A, pH 5.1; 9A, pH 5.8 and 11B, pH 6.0) the soluble nitrogen recovered is distinctly less than that assumed to be liberated. In soils 5B (pH 6.7) and 7B (pH 6.5)

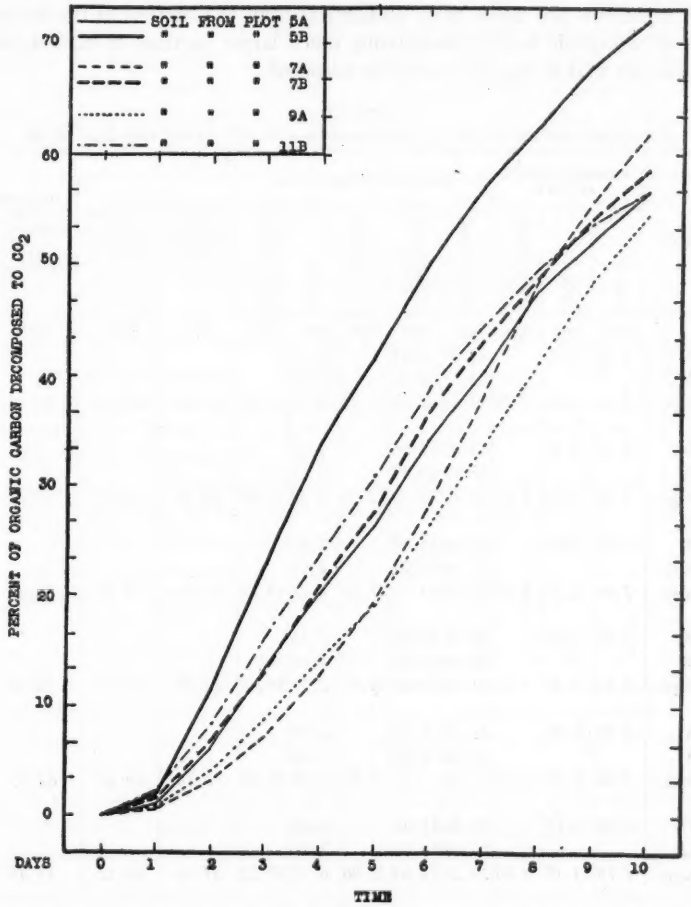

FIG. 5. COURSES OF DECOMPOSITION OF DRIED BLOOD IN SOILS OF DIFFERENT FERTILITY

there are scarcely any differences between the amounts of soluble nitrogen recovered and those calculated to have been liberated. This can be explained by a proper understanding of the organisms affecting the decomposition in the different instances. Although bacteria were very active in all cases, the fungi made particularly abundant development in the more acid soils and the

actinomycetes also developed in the soils later due to the favorable alkaline reaction created by the ammonia. In the more nearly neutral soils the fungi made a comparatively scant development and the decomposition was effected almost entirely by the bacteria. The deficiencies in soluble nitrogen in the acid soils are accounted for in the amounts assimilated by the fungi, since these organisms are much more economical than the bacteria in the decomposition of organic matter assimilating much larger portions of carbon and nitrogen per unit of organic matter decomposed.

TABLE 7

Ammonium and nitrate nitrogen transformations in soils treated with dried blood

SOIL NUMBER	NITROGEN CONTENT AT START			NITROGEN CONTENT AT END					INCREASE IN SOLUBLE N	N ADDED IN ORGANIC MATTER	CALCULATED N IN AMOUNT ORGANIC MATTER DECOMPOSED
	NH_3-N	NO_3-N	Total soluble N	NH_3-N in soil	NH_3-N in air stream	Total NH_3-N	NO_3-N	Total soluble N			
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
5A	2.24	3.68		32.39	2.93		6.91				
5A				34.88	4.11		5.99				
Average..	*2.24*	*3.68*	*5.92*	*33.64*	*3.52*	*37.16*	*6.45*	*43.61*	*37.69*	*96.12*	*54.55*
5B	1.79	3.78		20.07	54.86		2.25				
5B				19.77	50.17		2.81				
Average..	*1.79*	*3.78*	*5.57*	*19.92*	*52.52*	*72.44*	*2.53*	*74.97*	*69.40*	*96.12*	*69.28*
7A	1.49	3.27		33.56	14.37		2.50				
7A				34.00	13.20		2.05				
Average..	*1.49*	*3.27*	*4.76*	*33.78*	*13.79*	*47.57*	*2.28*	*49.85*	*45.09*	*96.12*	*59.46*
7B	1.64	3.59		20.87	35.20		2.75				
7B				21.39	36.08		2.66				
Average..	*1.64*	*3.59*	*5.23*	*21.13*	*35.64*	*56.77*	*2.71*	*59.48*	*54.25*	*96.12*	*56.19*
9A	2.09	0.99		32.31	4.25		3.99				
9A				32.24	3.08		3.99				
Average..	*2.09*	*0.99*	*3.08*	*32.28*	*3.67*	*35.95*	*3.99*	*39.94*	*36.86*	*96.12*	*52.35*
11B	1.79	3.17		19.48	11.44		6.65				
11B				19.04	15.84		5.99				
Average..	*1.79*	*3.17*	*4.96*	*19.26*	*13.64*	*32.90*	*6.32*	*39.22*	*34.26*	*96.12*	*54.49*

Figures 3–5 exhibit largely the same characteristics in the different soils as those shown previously for the decomposition in one soil. The differences appear to be largely quantitative and not qualitative.

From these and other results (27) the further conclusion seems justified that the differences in the carbon dioxide produced from the carbon in the soils without other additions of organic matter are much greater than the differences in carbon dioxide produced by these same soils from added organic matter.

DISCUSSION

Contrary to the opinions expressed by Whiting and Schoonover that production of carbon dioxide was not as good an index of the decomposition as nitrate-nitrogen accumulation, the author has found the measurement of carbon dioxide very satisfactory. The fact as stated that there is no true relation between the carbon oxidized and the nitrogen nitrified speaks rather against the measurement of nitrate nitrogen. Their results on the amounts of clover decomposed for different periods as indicated by nitrate accumulation are decidedly lower than those obtained by many other workers using production of carbon dioxide. It is further apparent from the results recorded in this paper and particularly those recently presented by Lyon, Bizzell and Wilson (11) that the accumulation of soluble nitrogen is not proportional to the decomposition of clover and alfalfa. This is largely due to the fact that the nitrogen becomes practically all temporarily assimilated into the microbial bodies during the decomposition of materials containing about 2 per cent nitrogen. Further, the kinds of organisms active in the decomposition as regulated by the soil conditions will greatly affect the amount of nitrogen liberated and reassimilated. Besides the greater accuracy of the method for determining organic matter decomposition. the rate of the decomposition may more easily be followed by measuring carbon dioxide evolved than by determining nitrate accumulation.

The rapidity of decomposition of some crude organic materials such as rye and alfalfa may be associated somewhat with their nitrogenous composition. However, nitrogen is far from being the only limiting factor. Some organic materials decompose easily and others with much more difficulty irrespective of their nitrogenous contents. As an example, dextrose and cellulose may be cited. These two compounds both entirely lack nitrogen. One, the dextrose, decomposed the most readily of all the materials studied, even those very rich in nitrogen; the other, cellulose, decomposed the slowest of all those considered. Apparently no broad generalizations apply to the relative ease of decomposition on the basis of carbon, nitrogen nor oxygen contents. It is probably true, however, that the simpler organic materials, due to the readiness with which they are attacked by all lower organisms do decompose most readily, while the more complex ones, by reason of the few organisms that can attack them are decomposed more slowly.

SUMMARY

The decomposition of cellulose, dextrose, rye straw, alfalfa meal, dried blood, and mixed spore and mycelium material of fungi has been followed in the same soil and, in some cases, in soils of different fertility by determining the carbon dioxide evolved. The results lead to the following conclusions:

1. The carbon dioxide evolved from decomposing organic matter serves as a reliable index of the process of decomposition.

2. In the same soil, the amount of decomposition of different organic materials at any one time is different.

3. Decomposition of the organic materials is very rapid in fertile soils. After two days, 35 per cent of the carbon in dextrose had been given off as carbon dioxide, 26 per cent of the alfalfa meal, 19 per cent of the fungous material, 16 per cent of the rye straw, 7 per cent of the dried blood, and 0.5 per cent of the cellulose. The decomposition after ten days was 68 per cent of the dried blood, 67 per cent of the fungous material, 55 per cent of the alfalfa meal, 36 per cent of the rye straw, and 20 per cent of the cellulose. It is noteworthy that fungous material decomposes very rapidly.

4. During the first few days the differences in decomposition of the different materials are greatest.

5. In general, the period of maximum decomposition was one of the first few days after adding the material to the soil.

6. The rate with which each organic material decomposes is distinctive, some decomposing rapidly from the start, others showing a decided lag in the early stages of decomposition, and still others showing a uniformly moderate decomposition.

7. Cellulose decomposes most slowly, followed by the rye straw, in turn followed by alfalfa meal, fungous material and dried blood. Dextrose decomposes the most rapidly of all the materials used.

8. Nitrate distinctly accelerated decomposition of rye straw and cellulose but was inaffective when added to alfalfa meal. The effect of the nitrate on straw decomposition was noted on the third day. It continued to hasten decomposition to the end of the period.

9. Rye straw tends to deplete the soluble nitrogen in the soil during the early stages of decomposition; alfalfa meal slightly increases it; and dried blood greatly increases it, particularly the ammonia. These effects are correlated with the nitrogen contents of the organic materials.

10. The decomposition of the same material in soils of different fertility is different, but the order of the soils with respect to the rapidity with which they decompose one organic material is not the same for another. In general, however, fertile soils decompose organic matter more rapidly than those less productive and soils nearly neutral decompose organic matter more rapidly than those more acid.

11. The differences in production of carbon dioxide from the carbon contained in soils without further additions of organic matter are much greater than the differences in carbon dioxide produced from these same soils from added organic matter. The decomposing powers of different soils for most organic materials do not vary greatly.

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SORGHUM AS AN INDICATOR OF AVAILABLE SOIL-NITROGEN

R. M. PINCKNEY

Minnesota Agricultural Experiment Station

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In an earlier paper (1) the writer has described greenhouse experiments showing that the hydrocyanic acid content of sorghum plants increases with the amount of nitrate applied, and has suggested the use of sorghum as an indicator plant in studies on the readily available nitrogen in soils. The present paper reports a trial of this suggestion under field conditions. The method used for the determination of the hydrocyanic acid has been the same as that described in the earlier paper.

PLOTS ON UNIVERSITY FARM

On June 12, 1923, sorghum was sown thickly in drills six inches apart, in two fields on the Minnesota Experiment Station Farm. Both fields had a fertile soil. Site I had been in a rotation including legumes, while site II had been continuously in legumes for several years. Six days later, when the plants were well up, plots one rod square were marked off, and sodium nitrate was scattered over them, at the rates of 0, 200, 400, 800, and 1600 pounds per acre.

Five days after the nitrate had been applied, two inches of rain fell (table 1), and there was some flooding, and probably some of the nitrate was washed away. In the case of site II, additional plots were marked off and nitrate applied on June 29 as before, taking care to use for these only portions of the field that were too high to have received any run-off from the previously fertilized plots.

Table 1 shows the rainfall during the time of the experiment.

Samples were taken and the prussic acid determined, first on June 29, next on August 6, and lastly on September 4 (tables 2, 3, and 4).

On June 29 all the plots that had received nitrate presented a spotted appearance, showing some stunted and some dead plants, the number of the latter increasing with the amount of nitrate applied. These spots were no doubt due to excessive amounts of nitrate, as the application by hand could not be made entirely uniform, thus producing an "alkali" effect upon the young plants, stunting them for a time, or killing them outright where the nitrate solution became too strong. The prussic acid content on June 29 is given in table 2. The transitory stunting effect of the nitrate is best shown in the weight of plants from site II, the plants on all the fertilized plots at that site being smaller than on the unfertilized plot.

At the second sampling (table 3) the height of the plants with the leaves stretched upward was measured, and the weight of the green plants per square meter was determined, cutting them as low as possible. In the plants from plot 2, site I, the percentage of dry matter also was determined, and found to be 13.35 per cent.

TABLE 1

Rainfall at University Farm from June 1, to September 3, 1923

JUNE		JUNE		JULY		AUGUST	
Date	Amount	Date	Amount	Date	Amount	Date	Amount
	inches		*inches*		*inches*		*inches*
4	0.20	20	0.05	5	0.11	2	0.13
5	0.02	23	0.22	10	0.45	8	0.17
7	0.56	24	0.17	11	0.16	11	0.21
13	0.45	25	2.00	27	0.87	21	1.31
18	0.56	26	0.07	29	0.35	28	0.08
19	0.60	28	0.05	30	0.96		
Totals.....		4.95		2.90		1.90	
Total for the period.....						9.75	

TABLE 2

Hydrocyanic acid found on June 29 in sorghum plants 17 days after seeding

PLOT	NITRATE APPLIED		AVERAGE HEIGHT OF PLANTS	AVERAGE WEIGHT OF PLANTS	HYDROCYANIC ACID (HCN) FOUND PER PLANT	
	Per plot	Per acre				
	lbs.	*lbs.*	*cm.*	*gm.*	*per cent*	*mgm.*
			Plots on Site I			
1	None	None	11	0.155	0.30	0.465
2	1.25	200	14	0.232	0.27	0.628
3	2.50	400	14	0.188	0.28	0.528
4	5.00	800	14	0.185	0.50	0.926
5	10.00	1600	14	0.200	0.42	0.840
			Plots on Site II			
6	None	None	18	0.490	0.30	1.470
7	1.25	200	15	0.322	0.50	1.610
8	2.50	400	14	0.262	0.53	1.390
9	5.00	800	13	0.233	0.39	0.910
10	10.00	1600	15	0.200	0.54	1.080

The data show that on university farm soil, which is well supplied with available nitrogen, the addition of sodium nitrate increased the yield of green sorghum from 15 to 80 per cent, and the hydrocyanic acid in the green material still more. Since the first analysis, thirty-eight days before, the hydrocyanic acid *per plant* on the unfertilized plots had diminished by about one half,

while it had *increased* on six of the eight fertilized plots, and in general had increased more on the heavily fertilized than on the lightly fertilized ones.

TABLE 3

Relative growth and hydrocyanic acid found in sorghum plants on August 6, 35 days after seeding

PLOT NUMBER	NITRATE APPLIED PER ACRE	AVERAGE HEIGHT OF PLANTS	WEIGHT OF GREEN SORGHUM PER SQUARE METER	AVERAGE WEIGHT OF PLANTS	HYDROCYANIC ACID (HCN) FOUND			
					In leaves	In stems	In whole plant	Per whole plant
	lbs.	*cm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*mgm.*
				Plots on Site I				
1	None	91	2160	6	0.0069	0.0016	0.0035	0.210
2	200	122	3200	19	0.0076	0.0038	0.0045	0.856
3	400	122	3420	25	0.0150	0.0014	0.0079	1.975
4	800	113	3035	25	0.0120	0.0034	0.0063	1.575
5	1600	113	3905	26	0.0130	0.0051	0.0077	2.002
				Plots on Site II				
6	None	152	2990	18	0.0083	0.0017	0.0040	0.720
7	200	165	3520	20	0.0078	0.0021	0.0040	0.800
8	400	167	3580	17	0.0115	0.0035	0.0065	1.105
9	800	172	3740	42	0.0177	0.0029	0.0078	3.280
10	1600	178	3470	25	0.0174	0.0024	0.0078	1.950

TABLE 4

Hydrocyanic acid found on September 4 in sorghum plants 88 days after seeding

PLOT	NITRATE APPLIED PER ACRE	AVERAGE WEIGHT OF PLANTS	HYDROCYANIC ACID (HCN) FOUND			
			In leaves	In stems	In whole plant	Per whole plant
	lbs.	*gm.*	*per cent*	*per cent*	*per cent*	*mgm.*
			Site I			
1	None	29	Trace	Trace	Trace	Trace
2	200	37	0.00018	0.00006	0.00009	0.033
3	400	30	0.00030	0.00026	0.00027	0.083
4	800	28	0.00550	0.00260	0.00330	0.924
5	1600	27	0.00490	0.00260	0.00320	0.864
			Site II			
6	None	26	0.00550	0.00140	0.00180	0.468
7	200	36	0.00850	0.00070	0.00310	1.116
8	400	32	0.00620	0.00120	0.00250	0.800
9	800	58	0.00460	0.00120	0.00190	1.102
10	1600	46	0.00720	0.00130	0.00340	1.564

It should be pointed out, however, that on two of the fertilized plots the weight of hydrocyanic acid per plant had diminished, and that there is no

distinct parallel between the amount of nitrate added and the amount of hydrocyanic per plant found. The percentage of prussic acid at the time of the second analysis was in general only about one hundredth of what it had been thirty-eight days before. This rapid diminution in the percentage of prussic acid in the growing plants makes it imperative in most cases to compare only plants of the same *age*. It is possible that due to the irregular distribution of the nitrate on the ground, some of the plants that show unduly large amounts of prussic acid may have grown on spots where lumps of nitrate fell.

The analyses of September 4 (table 4) agree with the earlier ones (tables 2 and 3) in showing that there is more prussic acid in plants that were given

TABLE 5

Percentage and amount per plant of hydrocyanic acid found in sorghum plants at different ages

PLOT	NITRATE APPLIED PER ACRE	HCN CONTENT AT END OF VARIOUS INTERVALS AFTER SEEDING					
		17 days		55 days		88 days	
	lbs.	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*
				Site I			
1	None	0.3000	0.465	0.0035	0.210	Trace	Trace
2	200	0.2700	0.628	0.0045	0.856	0.00029	0.033
3	400	0.2800	0.528	0.0079	1.975	0.00027	0.083
4	800	0.5000	0.926	0.0063	1.675	0.00330	0.924
5	1600	0.4200	0.840	0.0077	2.002	0.00320	0.864
				Site II			
6	None	0.3000	1.470	0.0040	0.720	0.00180	0.468
7	200	0.5000	1.610	0.0040	0.800	0.00310	1.116
8	400	0.5300	1.390	0.0065	1.105	0.00250	0.800
9	800	0.3900	0.910	0.0078	3.280	0.00190	1.102
10	1600	0.5400	1.080	0.0078	1.950	0.00340	1.564

nitrate than in the unfertilized ones, but the amount is only roughly in proportion to the amount of nitrate applied.

A comparison from tables 2, 3, and 4 is given in table 5. The data show that the percentage of hydrocyanic acid was greatest in very young plants and less at each succeeding sampling. From tables 3 and 4 it will be seen that the percentage of prussic acid was much greater in the leaf than in the stem and that it varied more in the leaf. The total amount per plant varied, in general, as the nitrate applied. On the unfertilized plots it diminished from first to last, but on six of the eight fertilized, it first increased, and later decreased.

Up to the September sampling, care had been taken that the samples analyzed should all be from the interior of the plots, in order to avoid the effect of applications on adjoining plots. At this sampling the plants along

the margin of the plots, where they had more room, and more especially along the margin of the no-nitrate plot, were larger and greener than those in the interior of the same plot. Such is usually the case when any crop is planted thickly enough to be crowded. It was to be expected that these differences were due to a more liberal supply of water or of nitrate or of both. The earlier results of these experiments suggested that the difference in nitrate would be reflected in a greater amount of prussic acid in the plants that grew along the margin and such was the result found by analysis (table 6, site I).

TABLE 6

Hydrocyanic acid found September 6 in sorghum from margin and interior of University Farm plots

SOURCE OF SAMPLE		HEIGHT	AVERAGE WEIGHT PER PLANT	HYDROCYANIC ACID FOUND			
				Leaf	Stem	Whole plant	Per whole plant
		inches	*gm.*	*per cent*	*per cent*	*per cent*	*mgm.*
Site I	Margin	52	50	0.00230	0.00160	0.00184	0.920
	interior	52	52	Trace	0.00180	0.00137	0.712
Site II	margin	70	126	0.00550	0.00140	0.00180	2.268
	interior	48	20	0.00830	0.00120	0.00376	0.752

SAMPLES FROM FIELDS ON OTHER FARMS

This result in turn suggested the analysis of similar pairs of samples from fields other than on University Farm, wherever they showed similar differences in growth and color. Several pairs were secured and analyzed, but none were grown under conditions that even approximate those sought. No fields were found where the sorghum had been sown broadcast, nor in drills close together, as on the plots on University Farm, so as to make the crowding and the resulting competition for nitrate severe.

On all, the sorghum had been planted in rows, 30 inches to 36 inches apart, and kept clean cultivated. The crowding, even where the plants were thickest, was far less pronounced than on the plots at University Farm, and accordingly the plants were in general taller, and much more stocky. In several fields examined, the plants at the margin instead of being more thrifty than in the interior were less so, due to weeds or grass, or in some cases to shade from adjoining groves or orchards. Such were avoided, though where the lack of vigor is due to competition for nitrate, the results should be the same as where the plants at the margin are more vigorous than those in the interior.

There was no clear evidence of nitrogen hunger in any case. Even the samples secured from the Anoka sandy field, which were rather yellowish in color, may have been suffering quite as much from lack of water as from nitrogen hunger.

The analyses indicate the range in hydrocyanic acid that may be expected on average farms, where the sorghum has been given plenty of room and good cultivation but no nitrate, and is approaching maturity.

TABLE 7

Hydrocyanic acid found in sorghum from various fields

Grown in rows and kept clean cultivated, but not fertilized. In each case sample *b* is from the thicker stand.

SOURCE OF SAMPLE		HEIGHT	AVERAGE WEIGHT PER PLANT	HYDROCYANIC ACID FOUND			
				Leaf	Stem	Whole plant	Per whole plant
		inches	*gm.*	*per cent*	*per cent*	*per cent*	*mgm.*
III. Anoka Co.	a	48	51	0.00042	0.00012	0.00017	0.087
	b	40	24	0.00100	0.00042	0.00055	0.132
VII. Hennepin Co.	a	50	57	0.01660	0.00070	0.00340	1.938
	b	52	43	0.00130	0.00050	0.00060	0.258
VIII. Hennepin Co.	a	69	92	0.01380	0.00060	0.00360	3.313
	b	68	97	0.01010	0.00190	0.00260	2.522
IX. Hennepin Co.	a	70	96	0.01110	Trace	0.00270	2.592
	b	70	98	0.00960	Trace	0.00230	2.254
V. Hennepin Co.	a	..	73	0.00086	0.00018	0.00028	2.044
	b	..	44	Trace	None	Trace	Trace

TABLE 8

Hydrocyanic acid found in sorghum plants near or upon peat compared with those not so situated

SOURCE OF SAMPLE	HEIGHT	AVERAGE WEIGHT PER PLANT	HYDROCYANIC ACID FOUND			
			Leaf	Stem	Whole plant	Per whole plant
	inches	*gm.*	*per cent*	*per cent*	*per cent*	*mgm.*
VI. Hennepin County, a	100	163	0.00490	0.00260	0.00310	5.053
X. Hennepin County, b	50	73	0.00990	0.00200	0.00370	2.701
IV. Ramsey County, c	...	135	0.01330	0.00120	0.00580	7.830
XI. Ramsey County, d	...	103	0.00880	0.00220	0.00360	3.708

a. Peat soil covered with about twelve inches of sandy loam.
b. Dark sandy loam fifty feet from VI.
c. About six feet from edge of bog.
d. About twelve feet from edge of bog.

SUMMARY

1. Analyses for hydrocyanic acid were made at three stages of growth, 17 days, 55 days, and 88 days after seeding, in the case of sorghum grown on plots on two productive fields, both without added nitrate, and with various amounts applied shortly after the young plants appeared.

2. At the first stage the plants from all the plots contained a very high percentage of hydrocyanic acid, which was highest in those from the fertilized plots.

3. At the second stage the plants on all the plots given nitrate were larger and of a darker green color than on the unfertilized plots, while they also carried a higher percentage and larger amount per plant of hydrocyanic acid.

4. At the third stage the effect of the added nitrate was still more marked both in the percentage of hydrocyanic acid and in the amount per plant.

5. The data from the three stages of growth show that the percentage of hydrocyanic acid diminished from first to last, and that at all stages it was greater on the fertilized than on the unfertilized plots. The amount per plant on the unfertilized plots decreased from first to last, while on the fertilized plots it first increased and later decreased.

6. Since the percentage of hydrocyanic acid in the leaves usually leads to the same conclusion as that in the whole plants, it may sometimes suffice to analyze the leaves only in the case of older plants. Usually the total weight per plant appears the more trustworthy basis for comparison, but it is still better to report both the percentage and the amount per plant.

REFERENCE

(1) PINCKNEY, R. M., 1924. Effect of nitrate applications upon the hydrocyanic acid content of sorghum. *In* Jour. Agr. Res. (soon to appear).

PRELIMINARY NOTE ON THE DISTRIBUTION OF NITRATES IN SOIL UNDER CORN CULTURE[1]

A. W. BLAIR AND A. L. PRINCE

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The nitrogen availability plots at this station which have been under a five-year rotation of corn, oats (two years), wheat and timothy for the past fifteen years, have already been studied with reference to the nitrate content of the soil, under different nitrogen treatments, and also at different seasons of the year (1).

The corn crop of 1923 offered an opportunity for a study of the nitrate content of the soil in the vicinity of the growing plant (about 4 or 5 inches from the hill) in comparison with the content of the soil in the middle of the row (about 20 or 21 inches from the hill). Unfortunately the work was not started until late in the season and it is therefore not possible, at this time, to report results obtained at different dates throughout the growing season.

The results, representing only one sampling made early in August from a number of plots, are interesting and indicate the need of further study along this line. These plots have received annual applications of fertilizers as follows:

Plot 4. Acid phosphate and muriate of potash.
Plot 5. Acid phosphate and muriate of potash, plus 16 tons of cow manure.
Plot 7. No fertilizer of any kind.
Plot 9. Acid phosphate, muriate of potash, and nitrate of soda at the rate of 320 pounds per acre.
Plot 11. Acid phosphate, muriate of potash, and ammonium sulfate equivalent to 320 pounds nitrate of soda per acre.
Plot 13. Acid phosphate, muriate of potash, and dried blood equivalent to 320 pounds nitrate of soda per acre.
Plot 17. Acid phosphate, muriate of potash, and rye straw at the rate of 4000 pounds per acre.
Plot 18. Acid phosphate, muriate of potash, and nitrate of soda (as above) plus 16 tons of cow manure per acre.
Plot 19. Acid phosphate and muriate of potash.

The plots of one section designated *A* have not been limed during the entire time, while the plots of the other section designated *B* have received

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liberal applications of lime in the carbonate form at intervals of five years beginning with the corn crop of 1908. The fertilizer treatment has been the same for both sections. Samples of soil were taken by cutting to a depth of about $6\frac{2}{3}$ inches, promptly dried and nitrates determined by the phenol-disulfonic acid method. The results of these determinations, in parts of nitrate per million, together with the yields of dry shelled corn in bushels per acre are shown in table 1.

For the unlimed section it is noteworthy that in every case the nitrates are higher in samples taken away from the growing corn than near it, the average for the former being 7.52 parts per million and for the latter 3.31 parts per million. For the limed section there is little difference between samples taken near the corn and away from it, the average for the former being 2.53 and for the latter 2.43 parts per million. In both cases the figures are distinctly lower than the corresponding figures for the unlimed section.

TABLE 1

The distribution of nitrates under corn—unlimed and limed plots

PLOT NUMBER	A. UNLIMED			B. LIMED		
	Shelled corn per acre	Nitrates		Shelled corn per acre	Nitrates	
		Near corn	Away from corn		Near corn	Away from corn
	lbs.	*p.p.m.*	*p.p.m.*	*lbs.*	*p.p.m.*	*p.p.m.*
4	1795	1.18	1.40	2600	1.28	1.44
5	2775	3.60	5.32	2383	.....	....
7	695	0.82	6.78	2248	1.12	1.22
9	2180	5.27	6.67	2250	1.79	1.28
11	758	1.52	12.76	2800	1.33	1.68
13	2603	1.28	13.28	2970	1.55	1.63
17	1295	0.88	1.28	2653	1.76	1.76
18	3175	14.40	19.04	3413	10.00	8.80
19	1650	0.80	1.12	2405	1.44	1.60
Average.	1881	3.31	7.52	2636	2.53	2.43

It is possible that a more limited root system may have been the cause of the higher nitrates in soil from the middle of the rows on the unlimed plots. The soil of these plots is distinctly acid, ranging from pH 4.6 to pH 5.6, and this would no doubt limit root development to some extent. An average yield of 1881 pounds of shelled corn per acre on these plots as against 2636 pounds per acre on the limed plots would tend to support this theory. The soil of the limed plots had a pH value of about 6.9–7.2.

The higher nitrate content in the middle of the row could hardly be attributed to the cultivation, since if this were the cause, then the same difference should have been noted on the limed plots (level cultivation was practiced). The larger yields of corn on the limed plots together with fairly close agreement between the nitrates in samples taken near the growing corn and

in the middle of the row, seem to suggest, as pointed out above, a healthier and more uniform root distribution throughout the soil on the limed area.

The high nitrate content found in plots 18A and 18B must be attributed to the heavy application of nitrogenous fertilizers which these plots have received. With heavy applications of manure annually for fifteen years, the soil has become well supplied with organic matter, a part of which is easily nitrifiable. Plots 4A and 19A receive no nitrogen and 17A receives rye straw only, and the low nitrate content of these plots together with the low crop yields indicates little readily nitrifiable organic matter. Plot 11A, although having a pH value of 4.6, shows a high nitrate content in the sample from the middle of the row, which indicates that nitrification was going on even in the presence of high acidity. The fairly high nitrate content of soil from 9A reflects the nitrate of soda treatment for this plot.

Nitrate determinations were also made on samples of soil from two other corn plots which previous to this year had been continuously in soybeans for ten years. One of these, plot *M*, has been limed at intervals of five years (omitted in 1923) and the other had received no lime during that period. The nitrate results and yields of shelled corn are as follows:

PLOT	SHELLED CORN PER ACRE	NITRATES NEAR CORN	NITRATES AWAY FROM CORN
	lbs.	*p.p.m.*	*p.p.m.*
M. Limed	2465	0.92	1.57
N. Not limed	308	6.12	13.32

Here as in the previous experiments nitrates are higher in samples taken from the middle of the row than in those taken near the corn. The soil of plot *N* is very acid, pH 4.6, and as a consequence the corn crop was practically a failure. Thus the small amount of nitrates required for the crop would seem to account for the rather high nitrate content of the soil.

Nitrates were determined in samples from two other corn plots, 65 and 66, both of which received nitrogenous fertilizers and were also limed. The nitrate results and yields of shelled corn were as follows:

PLOT	SHELLED CORN PER ACRE	NITRATES NEAR CORN	NITRATES AWAY FROM CORN
	lbs.	*p.p.m.*	*p.p.m.*
65. Limed	3000	0.72	1.13
66. Limed	2925	1.39	4.64

Here also the nitrates are higher in samples taken away from the corn than in those taken near it, but since both plots were limed the explanation given above for the difference does not hold here. However, with one exception it is apparent that the nitrates were being utilized almost up to the limit, and root development was probably normal in both cases.

Definite conclusions should not be drawn until further work is done, but there is good reason to suspect a more complete utilization of the nitrates that are formed in the soil, when the soil reaction is near the neutral point, than when it is distinctly on the acid side.

REFERENCE

(1) BLAIR, A. W., AND PRINCE, A. L. 1922 Variation of nitrate nitrogen and pH values of soils from the nitrogen availability plots. *In* Soil Sci., v. 14, no. 1, p. 9–19

INFLUENCE OF VARYING RATIOS OF PHOSPHORIC ACID AND POTASH ON CROP YIELD AND NITROGEN RECOVERY

A. W. BLAIR AND A. L. PRINCE

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In an earlier paper (1) it was pointed out that a nitrogen, phosphoric acid, potash ratio of 5-10-4 gave a slightly higher yield of barley (grain and straw) than a ratio of either 5-5-4 or 5-15-4. With a second or residual crop of sorghum, doubling and trebling the application of phosphoric acid gave no appreciable increase in yield. Under the same conditions doubling the potash application gave a slight increase in the barley crop but no appreciable increase in the residual crop.

The increased amounts of phosphoric acid and potash had little influence on the percentage of nitrogen and phosphoric acid in the crops. In most cases the higher amounts of phosphoric acid and potash did give somewhat higher nitrogen recoveries. For example, with a ratio of 5-5-4 the recovery of nitrogen from nitrate of soda was 61.2 per cent and with a ratio of 5-15-4 it was 82.3 per cent. With a ratio of 5-5-8 the recovery was 69 per cent and with a ratio of 5-15-8 it was 76.1 per cent.

A higher recovery of nitrogen was obtained when the nitrogen was in the form of nitrate of soda than when in the form of ammonium sulfate, dried blood, or a mixture of the three. Taking the two crops together, the lowest recovery was from ammonium sulfate, with an average of about 45 per cent, the dried blood and the mixture of the three materials standing between this and the nitrate of soda, with an average of a little more than 50 per cent.

RESULTS FOR THE CORN CROP OF 1923

The work which was started in 1922 was continued in 1923 without change in plan. The plan provides for sixty outdoor cylinders having a surface area of three square feet. The soil used is a silt loam which had not been under cultivation for many years and was distinctly acid. To overcome this acidity pulverized limestone was applied to all cylinders in amounts sufficient to bring the reaction to about pH 6.6–7.0. In applying the fertilizers, provision is made for nitrogen, phosphoric acid and potash ratios of 5-5-4; 5-10-4 and 5-15-4, and this is repeated with the amount of potash doubled. Nitrogen is used in the form of nitrate of soda, ammonium sulfate, dried blood and a com-

[1] Paper No. 169 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

bination of the three. Equivalent amounts of nitrogen are used in all cases. The work is carried out in duplicate.

In the fall of 1922 the soil was prepared and phosphoric acid and potash applied in accordance with the plan (nitrogenous fertilizers were withheld for spring application), and all cylinders were seeded to wheat. On account of winter injury the wheat was treated as a green manure crop and the ground prepared for corn in the spring of 1923. This was planted May 14 and the nitrogenous fertilizers applied in accordance with the plan. Phosphoric acid and potash were not applied at this time. The corn was planted thick to be harvested as forage and not as a mature crop. Growth through the season was normal and the corn was harvested August 16. This was thoroughly dried, weighed and samples prepared for analysis. The yields of dry matter and percentage of nitrogen and nitrogen recovered, for the various treatments, are shown in table 1.

Attention may be called to the fact that a larger average yield was obtained with the 5-10-4 ratio than with either the 5-5-4 or the 5-15-4; likewise the 5-10-8 ratio gave a larger yield than the 5-5-8 or the 5-15-8. It would thus appear that for the soil and crop in question, at least, an amount of phosphoric acid equal to twice the amount of nitrogen used gave better results than when the two were used in equal amounts or when the phosphoric acid was three times the amount of the nitrogen. This is interesting since it is about the ratio which obtains in a number of the high grade fertilizers, especially the potato fertilizers.

A study of the percentage of nitrogen found in the dry matter reveals the fact that this was not influenced by the amount of phosphoric acid used, the figures being practically the same, within the limit of error, for the three different amounts of phosphoric acid. It may be pointed out furthermore that the percentage of nitrogen varies within quite narrow limits—0.45 to 0.50 per cent. Taking the general average, it is found that the percentage of nitrogen is slightly higher with the double than with the single portion of potash, irrespective of the amount of phosphoric acid used.

Evidently the amount of phosphoric acid has had only slight influence on the percentage of nitrogen recovered. The highest average recovery, 34.5 per cent, was with the 5-15-4 ratio, and the next highest average, 32.6 per cent, was with the 5-10-8 ratio. The general average with the single portion of potash is about one point higher than the corresponding figure with the double portion of potash.

The form in which the nitrogen was used has had a far greater influence on the per cent recovered than has the amount of phosphoric acid or potash. For the section with the single portion of potash the average recovery with nitrate of soda was 41 per cent, and with dried blood 22 per cent. The corresponding figures for the section with the double portion of potash are 45.3 and 19 per cent respectively. The recoveries with ammonium sulfate, and with the combination of the three materials, stand between the nitrate of soda and the dried blood.

TABLE 1

Yield of dry matter (corn forage), per cent nitrogen in dry matter, and per cent nitrogen recovered

(N, P_2O_5, and K_2O used in ratios as indicated)

	Dry matter	Nitrogen in dry matter	Nitrogen recovered	Dry matter	Nitrogen in dry matter	Nitrogen recovered	Dry matter	Nitrogen in dry matter	Nitrogen recovered	Dry matter	Nitrogen in dry matter	Nitrogen recovered
	gm.	*per cent*	*per cent*	*gm.*	*per cent*	*per cent*	*gm.*	*per cent*	*per cent*	*gm.*	*per cent*	*per cent*
	RATIO 5-5-4			RATIO 5-10-4			RATIO 5-15-4			AVERAGE		
A. Check	140.8	0.462	...	138.6	0.510	...	136.5	0.490	...	138.6	0.488	...
B. Nitrate of soda	271.8	0.486	40.6	285.1	0.470	38.4	285.5	0.487	43.9	280.8	0.481	41.0
C. Ammonium sulfate	240.7	0.478	30.3	235.6	0.470	24.2	249.8	0.475	31.4	242.0	0.474	28.6
D. Dried blood	202.3	0.495	21.4	224.8	0.454	19.0	215.0	0.506	25.6	214.0	0.485	22.0
E. $\frac{1}{3}$ N from each	254.0	0.470	33.0	279.8	0.474	37.6	272.1	0.470	37.1	268.6	0.472	35.9
Average	*221.9*	*0.478*	*31.3*	*232.8*	*0.477*	*29.8*	*231.8*	*0.486*	*34.5*	*228.8*	*0.480*	*31.9*
	RATIO 5-5-8			RATIO 5-10-8			RATIO 5-15-8			AVERAGE		
F. Check	158.7	0.454	...	156.6	0.454	...	158.4	0.451	...	157.9	0.453	...
G. Nitrate of soda	308.5	0.470	44.2	321.0	0.451	44.7	316.3	0.470	46.9	315.3	0.464	45.3
H. Ammonium sulfate	265.0	0.451	28.7	267.0	0.462	31.8	274.3	0.451	31.7	268.8	0.455	30.7
I. Dried blood	217.0	0.467	17.6	239.5	0.443	21.3	213.3	0.474	18.0	223.3	0.462	19.0
J. $\frac{1}{3}$ N from each	241.4	0.454	22.7	269.0	0.464	32.7	263.3	0.454	29.2	257.9	0.457	28.2
Average	*238.1*	*0.459*	*28.3*	*250.6*	*0.455*	*32.6*	*245.1*	*0.460*	*31.5*	*244.6*	*0.458*	*30.8*

SUMMARY

Taking the work as a whole, a ratio of 1:2 for nitrogen and phosphoric acid gave somewhat better yields than a ratio of 1:1 or 1:3.

Neither the amount of phosphoric acid used nor the form of nitrogen have perceptibly influenced the percentage of nitrogen in the dry matter.

The form of nitrogen has on the other hand had a very pronounced influence on the crop yields and consequently on the percentage of nitrogen recovered in the crop. With nitrate of soda the recovery was, on an average, about 43 per cent; with ammonium sulfate it was close to 30 per cent and with dried blood a little over 20 per cent. When the nitrogen was taken from three sources (one-third from each) the recovery was, on the average, slightly better than with ammonium sulfate alone.

The recovery calculated on the basis of two crops, as in the case of 1922, is higher than with one crop only. This undoubtedly means a conservation of nitrogen, since much of what was taken up by the second crop would otherwise have been lost.

REFERENCE

(1) LIPMAN, J. G., AND BLAIR, A. W. 1923 The influence of varying ratios of phosphoric acid and potash on the yield, and on the nitrogen and phosphoric acid content of the crop, with different nitrogenous materials. *In* N. J. Agr. Exp. Sta. 44th Ann. Rpt. (soon to appear).

PLATE 1

NITRATE OF SODA WITH SINGLE AND DOUBLE PORTION OF POTASH

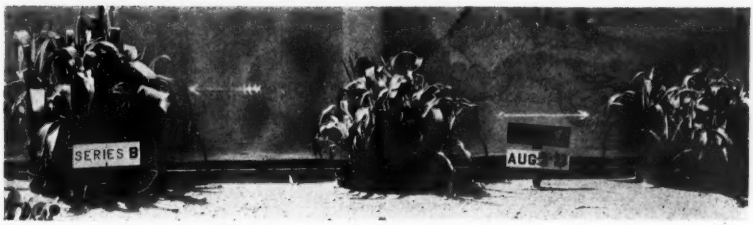

FIG. 1. TWO CYLINDERS UNDER SERIES A, NO NITROGEN; CYLINDER UNDER SERIES B, NITROGEN FROM NITRATE OF SODA

Ratio 5–15–4

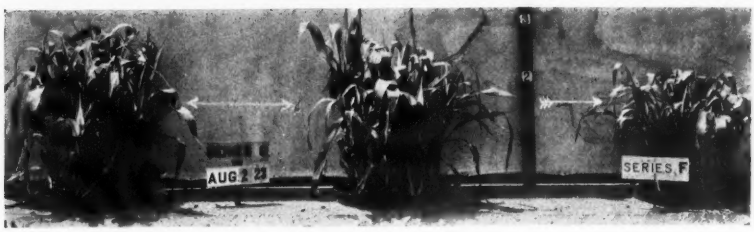

FIG. 2. CYLINDER UNDER SERIES F, NO NITROGEN; TWO CYLINDERS UNDER SERIES G, NITROGEN FROM NITRATE OF SODA

Ratio 5–15–8

NITRATES AND NITRIFICATION IN FIELD SOILS[1]

R. NAGAN GOWDA[2]

Iowa Agricultural Experiment Station

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The subject of nitrification in soil has been the theme of considerable investigation during the last thirty years and much information regarding the process has been accumulated. It has come to be quite generally recognized that there are certain conditions in the soil which to a large extent control the production of nitrates, or regulate the so-called nitrifying power. Thus it is believed that the requirements for the best nitrification in any soil include optimum moisture, plenty of easily decomposable organic matter, a basic reaction, good tilth, and the presence of available phosphorus and potassium. These are also the chief requirements for good crop yields and hence it would seem that there should be a relationship between the nitrates in soils or their nitrifying power and crop yields.

According to Brown (4) there is a rather close relation between bacterial activities and the crop-producing power of soils. Vogel's experiments (16) with potatoes and barley indicated that the productiveness of the soil was in direct relation to the nitrifying capacity. Burgess (5) considers nitrification by far the most accurate biological soil test for predicting the probable fertility of Hawaiian soils. But Jensen (8) thinks that the differences in the average seasonal accumulations of nitrates could not have been due entirely to the differences in yields of beets on the soils which he studied.

King and Whitson (9), Stewart and Greaves (15), Russel (14), Albrecht (1), and Whiting and Schoonover (20) found that the period of loss in nitrate content in soil in the summer coincided with the period of rapid growth of crops. Many investigators (9), (13), (7), (8) (14), (1), (20) have found that the greatest nitrification takes place in soil in the late spring and early summer. Several found an increase in nitrate content in the early autumn, following a period of low content in the late summer. Vogel (16), however, obtained the greatest nitrification in October and November and concluded that the treatment of the soil had less effect upon nitrification than the time of year. Lemmerman and Wichers (10) concluded that there is not sufficient evidence to show a direct periodic influence of the time of year on the life activities of the organisms and they believe that the physical factors control the process.

There seems to be no doubt that nitrification is affected very materially be variations in moisture content and investigations have placed the optimum at different points, ranging from 50 per cent up to 60 per cent saturation. The effects of manure, lime and phosphates on nitrification have been studied quite frequently and while the results vary considerably depending upon the conditions of the experiments and probably mainly upon the soil types used

[1] Part of the thesis submitted to the faculty of the Iowa State College in partial fulfullment of the requirements for the degree of Doctor of Philosophy.

[2] The author is indebted to Dr. P. E. Brown for advice in connection with this work and for reading the manuscript and to Dr. Paul Emerson for aid in the work.

in the tests, it has generally been found that these fertilizers give distinct and often large increases in the production of nitrates, and in the nitrifying power of the soil. The experiments of Warington (17), Withers (18), Brown (2), (3), Whiting and Schoonover (20), Noyes and Conner (12), Vogel (16), Jensen (8), White (19), and Fraps (6) give typical results with these fertilizers. Negative results have sometimes been secured as indicated in the work of Fraps (6), Vogel (16) and MacKenna (11), but these are exceptional cases.

EXPERIMENTAL

The purpose of the experiments reported here was to determine the effect of seasonal conditions, moisture content and various fertilizer treatments on

TABLE 1

Treatment of plots

PLOT NUMBER	TREATMENT
1300	Check
1301	1600 lbs. manure
1302	400 lbs. manure (annually)
1303	1600 lbs. manure + lime
1304	2400 lbs. manure + lime
1305	Check
1306	3200 lbs. manure + lime
1307	4000 lbs. manure + lime
1308	1600 lbs. manure + 200 lbs. raw rock phos. + lime
1311	1600 lbs. manure + 20 lbs. acid phosphate (ann.) + lime
1312	1600 lbs. manure + 200 lbs. raw rock phos. + 20 lbs. KCl (ann.) +lime
1313	1600 lbs. manure + 30 lbs. 2–8–2 (ann.) + lime
1314	1600 lbs. manure + 30 lbs. 2–12–5 (ann.) + lime
1315	Check
1324	Crop residues
1325	Check
1326	Crop residues + lime
1328	Crop residues + 200 lbs. raw rock phos. + lime
1331	Crop residues + 20 lbs. acid phosphate (ann.) + lime
1332	Crop residues + 30 lbs. 2–8–2 (ann.) + lime
1333	Crop residues + 200 lbs. raw rock phos. + 20 lbs. KCl (ann.) + lime
1334	Crop residues + 30 lbs. 2–12–5 (ann.) + lime.
1335	Check

the nitrate content and the nitrifying power of an Iowa soil and to ascertain if there is any relation to crop yields.

The experiment was carried out on some of the 36 plots in the 1300 series on the agronomy farm of the Iowa Agricultural Experiment Station. These plots are one-tenth of an acre in size, separated by division strips and surrounded by borders in the usual way and they have been under experiment for the past seven years. They are located on the Webster silty clay loam soil type, which is fairly uniform, but contains very small spots of the silt

loam and loam of the same series and of the Carrington loam and Clarion loam. The type is about as uniform as it occurs throughout the Wisconsin drift soil area.

The treatments to which these plots have been subjected are shown in table 1. A four-year rotation is practised and unless otherwise stated the treatments are made once in four years. In the year of this investigation

TABLE 2

Moisture determinations in soils

PLOT NUMBER	JUNE 21	JULY 25	AUGUST 9	AUGUST 30	SEPTEMBER 20*	SEPTEMBER 23	OCTOBER 12	AVERAGE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1300	17.5	7.5	6.5	12.5	20.0	17.5	16.0	13.9
1301	17.2	7.5	7.5	14.0	20.0	17.5	15.0	14.1
1302	15.0	13.5	6.5	13.0	20.0	17.5	18.0	14.7
1303	15.5	10.0	6.0	15.0	20.0	17.5	18.0	14.6
1304	17.0	9.0	7.5	12.5	20.0	17.5	18.0	14.5
1305	16.0	13.5	9.0	16.5	22.5	17.5	18.0	16.1
1306	14.5	12.5	10.0	16.5	22.5	20.0	21.0	16.7
1307	17.5	12.5	10.0	17.5	22.5	22.5	23.5	18.0
1308	17.5	15.0	14.0	17.5	20.0	22.5	20.0	18.1
1311	19.0	15.0	14.0	19.0	25.0	22.5	26.0	20.1
1312	19.0	15.0	16.5	17.5	20.0	20.0	25.0	19.0
1313	22.5	16.5	14.0	17.5	25.0	20.0	26.0	21.2
1314	22.2	18.5	17.5	21.0	25.0	27.5	27.5	22.8
1315	24.0	20.0	15.0	20.0	26.0	25.0	27.5	22.5
1324	17.5	12.5	11.0	15.0	20.5	20.0	20.0	16.5
1325	....	....	....	19.0	16.0	20.0	21.5	19.1
1326	14.0	11.5	13.0	19.0	20.0	17.5	26.0	17.3
1328	20.0	10.5	10.5	19.0	20.0	22.5	23.0	17.9
1331	19.5	16.5	12.5	18.5	25.0	25.0	20.0	19.5
1332	23.7	19.0	15.0	16.0	31.5	27.5	23.0	22.3
1333	21.2	19.0	11.5	17.0	25.0	25.0	18.0	18.1
1334	17.5	11.0	6.0	10.0	25.0	25.0	17.0	15.9
1335	15.5	10.0	5.0	9.0	25.0	20.0	13.0	13.9
Av.	18.3	12.5	10.8	16.2	22.5	21.2	21.0	

* Rained the previous day.

the plots were in corn. Clover had been grown in the preceding season and on plots 1324 to 1334 inclusive, the second cutting of clover was turned under for the residue treatment.

Eight samplings were made from these plots from June to October, each sample being taken from a different place in the plots. The surface soil was removed from an area two feet square and with a sterile trowel the soil in that area, to a depth of seven inches, was throughly mixed and the sample

was then drawn. Moisture determinations were made on all samples and the results are given in table 2. Nitrate determinations were made by the phenoldisulfonic acid method and the nitrifying power of the soils was determined by the beaker method using ammonium sulfate. The incubation period in the latter determination was four weeks at room temperature.

The nitrate content of the soils at the different samplings is shown in table 3. The highest amount in almost all cases was found in the samplings made in June. There was a gradual decrease during the next month and from then on the decrease was very rapid until the middle of September, when the amount of nitrate reached a minimum. Later a decided rise in the nitrate content occurred, as noted by others.

Comparing these results with those given for moisture in table 2, it seems that the most nitrates were present when the moisture content was about 18 per cent. The average moisture content for the whole field was about 18 per cent on June 21 when the nitrate content was the highest. Plots 1307, 1308, 1326 and 1328 which had a high nitrate content for the entire season had an average moisture content of about 18 per cent. Plots 1301, 1302 and 1304 which also contained considerable nitrates had an average moisture content of only 14 to 15 per cent but in general the best results were secured with the higher per cent. The results obtained on August 9 are significant. The rapid decrease in the nitrate content on that date coincided with the decrease in the moisture content which averaged 11 per cent for the whole series. This would indicate that the influence of the time of the year on nitrification is due particularly to high or low rainfall. Plot 1335 which throughout the season had in general a higher amount of nitrate than the adjacent plots 1334 and 1333 showed smaller amounts on August 9 and 30 when its moisture content was also lower. A comparison of the nitrates in the soils on September 20 and 23 is of interest. There was a heavy rainfall on September 19 and its effect is shown in the smaller amounts of nitrates on the next day.

Considering the effect of fertilization, it is evident that the addition of 8 tons of manure once in four years (plot 1301) or in four annual applications (plot 1302) caused an increase of about 60 per cent over plot 1305 in the nitrate content. The 12-ton application (plot 1304) caused a still greater increase in nitrates. Applications of manure larger than 12 tons did not cause as large increases.

The results on plot 1303 receiving 8 tons of manure cannot be explained with the data at hand. Evidently some unknown factor came into play there.

The year previous to this investigation plots 1324–1335 were in clover and the second cutting was turned under in the fall. Plot 1326 which received lime beside the clover turned under showed a 50 per cent increase in nitrates over the check plot 1325. In plot 1324 which received clover but no lime, nitrification was depressed. Probably the acids produced by the

decomposing green manure were not neutralized and therefore had an adverse effect on the nitrifying organisms.

Among the mineral fertilizer treatments rock phosphate (plots 1308 and 1328) resulted in the largest increase in nitrate nitrogen.

Table 4 shows the nitrifying power of the soils or their ability to serve as a medium for the growth of nitrifying organisms. The extent of the growth of nitrifying organisms is measured by the amount of $(NH_4)_2SO_4$ oxidized in a given period under optimum conditions of moisture and temperature.

TABLE 3

Pounds per acre of nitrate nitrogen in the surface soil in corn plots

PLOT NUMBER	JUNE 21	JULY 25	AUGUST 9	AUGUST 30	SEPTEMBER 20	SEPTEMBER 23	OCTOBER 12	AVERAGE
1300	77.62	56.04	22.84	16.18	11.78	10.42	15.24	30.02
1301	68.74	49.22	25.68	23.26	12.20	22.04	17.40	31.22
1302	67.52	81.30	24.98	12.84	8.18	7.76	16.34	31.28
1303	55.66	39.82	22.56	14.70	14.70	7.42	16.34	23.52
1304	90.50	45.88	21.88	14.98	11.50	10.76	22.94	34.06
1305	52.48	31.62	14.00	12.46	7.54	7.46	11.94	19.64
1306	46.88	36.58	15.02	19.74	8.06	6.78	18.74	21.74
1307	54.04	34.84	16.00	19.78	11.68	12.00	17.78	25.14
1308	45.82	42.26	20.66	22.58	7.96	8.04	24.00	24.48
1311	49.02	29.74	16.66	8.50	11.21	10.80	12.44	19.78
1312	41.98	48.92	18.78	11.06	8.06	12.20	17.34	22.65
1313	64.88	57.98	22.98	26.86	10.34	22.72	13.40	31.30
1314	95.70	32.68	20.78	16.30	11.74	12.40	22.34	30.26
1315	68.90	71.00	26.44	21.50	11.86	15.90	21.52	33.88
1324	32.78	22.22	13.12	11.60	8.00	11.10	15.76	16.38
1325				25.58	6.82	12.98	13.76	14.78
1326	50.56	46.10	13.04	18.48	7.28	11.98	18.66	23.72
1328	74.10	38.08	11.26	11.26	8.86	8.48	12.78	23.60
1331	49.04	36.78	18.48	13.48	9.44	6.58	11.50	20.76
1332	63.18	19.36	20.04	7.72	10.52	7.88	12.20	20.12
1333	51.66	27.16	11.58	9.14	7.62	8.20	10.98	18.04
1334	57.62	36.76	10.32	8.54	6.80	11.84	14.20	20.98
1335	79.90	40.34	9.52	7.40	11.60	10.88	12.20	24.54
Av.	61.28	44.02	16.98	16.88	10.32	12.28	17.42	

In general it is apparent that all the plots receiving treatments showed a higher nitrifying power than the untreated plots. The manure treatments up to 12 tons per acre caused large increases in nitrifying power. Sixteen- and twenty-ton applications caused small increases. The plots receiving crop residues together with mineral fertilizers showed the maximum nitrifying power. The plots receiving acid phosphate were higher in nitrifying power than those receiving rock phosphate. The more even distribution of the

acid phosphate in the soil may account for greater bacterial growth, but probably the solubility of the phosphorus is the more important factor.

In table 5, column 2 gives actual corn yield for 1921; column 3 gives calculated yields for all plots if they were untreated. This calculation is based on the assumption that there is a gradual variation in the natural productiveness of the soil. According to the yields of check plots 1305 and 1315 there is a progressive reduction in the natural productiveness of these and the intervening plots. The difference of 900 lbs. in yield between these plots is therefore evenly distributed over all the plots in between. Similarly with the gradual increase from plot 1315 to 1325 and from 1325 to 1335. Column 4 gives the difference between columns 2 and 3 or the increase due to treatment over the calculated unaided yield. In column 5 the same is expressed in percentages. Up to and including 20 tons, the larger the amount of manure applied the greater was the crop yield obtained. The 20-ton

TABLE 4

The nitrification of $(NH_4)_2SO_4$ calculated as pounds per acre of nitrate nitrogen in the surface soil in corn plots variously treated

PLOT NUMBER	JUNE 21	JULY 12	JULY 25	AUGUST 9	AUGUST 20	SEPTEMBER 20	SEPTEMBER 22	OCTOBER 12	AVERAGE
1300	532.4	443.6	276.2	426.8	351.6	763.6	299.2	315.2	426.0
1301	514.8	474.4	286.0	498.8	332.6	441.2	401.8	314.8	408.0
1302	380.4	454.8	344.8	414.0	374.0	569.6	398.8	337.8	408.0
1303	406.2	456.2	330.8	440.4	364.2	288.0	314.8	210.0	351.4
1304	575.4	454.0	328.0	456.6	297.0	531.0	341.6	167.4	393.8
1305	427.2	405.0	296.0	329.6	321.9	226.6	204.8	131.4	292.8
1306	504.6	438.2	318.0	404.0	333.4	326.0	359.8	134.4	341.0
1307	491.6	554.0	291.4	325.4	293.0	609.2	281.6	192.2	379.8
1308	513.4	430.4	293.0	489.8	360.0	467.6	259.8	269.4	385.4
1311	578.6	465.4	370.6	489.8	231.0	824.0	399.0	294.8	481.6
1312	472.6	484.2	313.4	504.4	408.0	756.8	365.8	343.0	456.0
1313	607.0	523.6	352.0	498.0	352.6	844.4	392.8	313.8	485.6
1314	566.4	493.6	279.8	421.0	383.4	872.8	414.2	301.0	466.6
1315	697.2	512.4	290.2	448.2	398.0	794.6	375.4	132.6	456.0
1324	336.0	444.4	218.0	379.6	309.4	277.8	252.6	187.6	300.6
1325					274.0	311.8	233.0	125.6	236.1
1326	395.6	335.6	219.6	379.4	294.8	400.0	305.8	118.0	306.1
1328	384.6	394.6	233.2	442.4	370.4	455.8	225.8	326.8	366.6
1331	534.4	502.0	306.0	377.2	310.2	826.6	375.4	290.2	440.2
1332	608.2	505.8	262.0	487.0	289.2	858.4	390.4	249.4	456.2
1333	528.8	434.0	260.0	404.0	265.0	923.2	390.2	168.8	421.8
1334	406.4	483.8	230.0	398.8	318.4	276.4	251.4	170.0	304.9
1335	384.8	395.4	193.2	476.8	154.0	311.6	281.8	129.2	290.8
Av.	496.6	453.8	286.0	432.0	329.8	562.6	326.0	227.0	

application effected an increase of 35 per cent and the 16-ton application, a 21.5 per cent increase while 12- and 8-ton applications resulted in increases of 14.9 and 6.4 per cent respectively. It would appear from this that the 20-ton application was the most efficient.

In the manure plots, rock phosphate with potassium caused as large an increase in crop yield as did acid phosphate. In the crop residue plots rock phosphate alone caused as large an increase as acid phosphate.

TABLE 5

Corn yields

PLOT NUMBER	CORN YIELD 1921 PER ACRE	CALCULATED YIELD*	INCREASE DUE TO TREATMENT	
	lbs.	*lbs.*	*lbs.*	*per cent*
1300	4700	4700	....	.....
1301	5000	4700	300	6.383
1302	4800	4700	100	2.127
1303	4800	4700	100	2.127
1304	5400	4700	700	14.89
1305	4700	4700	....	.....
1306	5600	4610	990	21.47
1307	6100	4520	1580	34.96
1308	5400	4430	970	41.90
1311	5600	4160	1440	34.61
1312	5500	4070	1430	35.14
1313	5600	3980	1520	38.19
1314	6000	3890	2110	53.99
1315	3800	3800	....	.....
1324	4190	3932	258	6.531
1325	3920	3920	....	.....
1326	4080	3961	119	3.004
1328	4430	4043	387	9.572
1331	4590	4166	424	10.181
1332	4460	4207	253	6.015
1333	4490	4248	242	5.695
1334	4640	4289	351	8.164
1335	4330	4330	....	.....

*As if all plots were untreated.

Commercial fertilizers applied with manure resulted in the largest increases in crop yield of all treatments. With crop residues, however, the effect of commercial fertilizers was not marked. Crop residues alone in the case of plot 1324 caused almost as great an increase in crop yield as plots 1332 and 1334 which received commercial fertilizers along with crop residues.

Figure 1 shows graphically the percentage increase or decrease in nitrate content, nitrifying power, and the crop yield (1921) induced by the treatments. The figures for nitrates and nitrifying power represent averages of the results obtained from seven samplings.

The curves for plots 1301, 1302, 1304, 1326 and 1328 indicate that the crop yield was proportional to the nitrate content. The curves for plots 1306, 1307, 1308, 1311, 1312 and 1314 apparently contradict the above statement but in reality they do not. The large increase in crop yield drew heavily on the nitrate content and hence the low nitrate content obtained.

The nitrate content of the soils seems to be proportional to their nitrifying power. This is indicated clearly in the curves for plots 1301, 1302, 1304, 1306, 1307, 1308, 1326 and 1328. The apparent discrepancy between plots 1311 and 1312 is explained as before by the factor of high crop yield and in plots 1331 and 1332 the high nitrifying power and low nitrate content is explained by the high moisture content during the period of sampling which

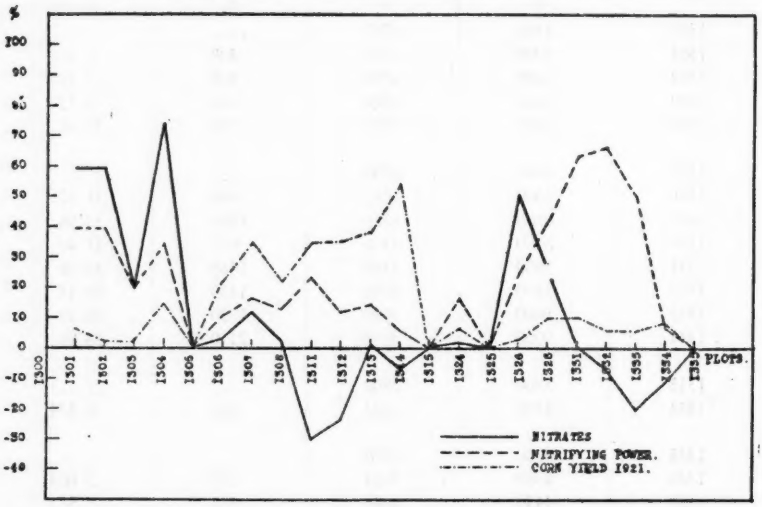

FIG. 1. INCREASE IN NITRATES, NITRIFYING POWER AND CORN YIELD DUE TO TREATMENT

has leached some of the nitrates, also by the fact that the crop yield on these plots was higher than on the check plot.

SUMMARY

1. In all the plots examined there was a large accumulation of nitrates in June with a gradual decrease in July, and a rapid decrease in August and September when it reached the minimum. In October there was a slight increase in nitrates.

2. The optimum moisture content for nitrification in this field soil was about 18 per cent.

3. The application of manure up to 12 tons per acre caused the greatest increase in nitrate accumulation and nitrifying power over the untreated soil. Sixteen- and twenty-ton applications caused smaller increases than those secured when 12 tons per acre were added.

4. The largest application of manure, 20 tons per acre, effected the greatest increase in crop yield in 1921 which was 35 per cent over the untreated plot. The 16-ton application caused an increase of 21 per cent over the untreated plot, the 12-ton application, 15 per cent; and 8-ton application, 6.4 per cent.

5. The crop residues turned under the previous fall increased the nitrate content of the soil when lime was present. The increase in nitrifying power due to the crop residues was also large. Crop residues increased the crop yield 6.5 per cent over the check.

6. Rock phosphate caused greater accumulation of nitrates in the soil than did either acid phosphate or commercial fertilizers. Rock phosphate with crop residues resulted in about as large an increase in crop yield as did acid phosphate.

7. Acid phosphate increased the nitrifying power of the soil more than the rock phosphate did.

8. Complete commercial fertilizers along with crop residues did not affect any greater increase in crop yield than did the crop residues alone.

9. The crop yield on this soil had a direct relation to the nitrate content and to the nitrifying power of the soil.

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SOIL VARIABILITY AS DETERMINED BY STATISTICAL METHODS[1]

ARTHUR H. POST

State College of Washington

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INTRODUCTION

It is recognized by most investigators that the soil constituents are variable for any given area. In the past, several methods have been used in sampling soil for chemical analysis. Probably the most common method was the use of a composite sample taken from any given area. This method may give a fairly uniform sample, but fails to show the soil variability.

The object of this paper was to determine, if possible, the variability of nitrogen in the soil, thereby giving one a closer check on fertility experiments.

REVIEW OF LITERATURE

Literature on the subject of soil variability and the probable error in sampling soil for chemical analysis is very limited. Most writers acknowledge the fact that single samples from any given area are of little value experimentally.

Russell (8) recommends 1 to 8 borings, depending on the uniformity of the soil to be tested. Hopkins and Pettit (6) advise the taking of borings from twenty or more places a few rods apart, and to mix thoroughly and use as a composite sample. Bear and Slater (4) compiled the methods used at the New York, Illinois and Wisconsin stations and recommend the following composite samples: A = 0 to 6⅔ inches, B = 6⅔ to 20 inches, and C = 20 to 40 inches; with 20 to 30 borings for A, and 10 to 15 borings for B and C. This would give good representative samples, but does not show the soil variability of the area sampled. Bear and McClure (3) recommend a 20-sample composite for a $\frac{1}{10}$ acre plot to a depth of 12 inches as giving a very uniform sample.

Robinson and Lloyd (7) determined the probable error in sampling soil for soil survey purposes and concluded that as a general rule the field error for chemical analysis is ±10 per cent, and for mechanical analysis is ±5 per cent. By making six determinations on a single sample of soil, they obtained a laboratory error of ±2.5 per cent. They believe that for soil survey purposes, six borings for a composite sample are satisfactory, where two analyses are made on the sample. Pfeffer and Blanck of Germany, cited by Robinson and Lloyd (7) in working on the nitrogen content of an experimental field, obtained a field error of 2.5 per cent. This, however, was only over a small area or plot. Waynick (9), in making a statistical study on nitrification, states that it is generally conceded among soil investigators that different samples of the same soil type from a comparatively limited area may show considerable

[1] Part of a thesis submitted to the Faculty of the State College of Washington in partial fulfillment of the requirements for a Master's degree in Agriculture.

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variation among themselves if we apply quantitative measurements to the various constituents of the soil mass. Eighty-one surface and subsurface samples of soil were taken and analyzed individually for nitrate, giving a field error of ±1.5 per cent for surface and ±3.1 per cent for the subsurface samples, this probable error being due to soil variability and field

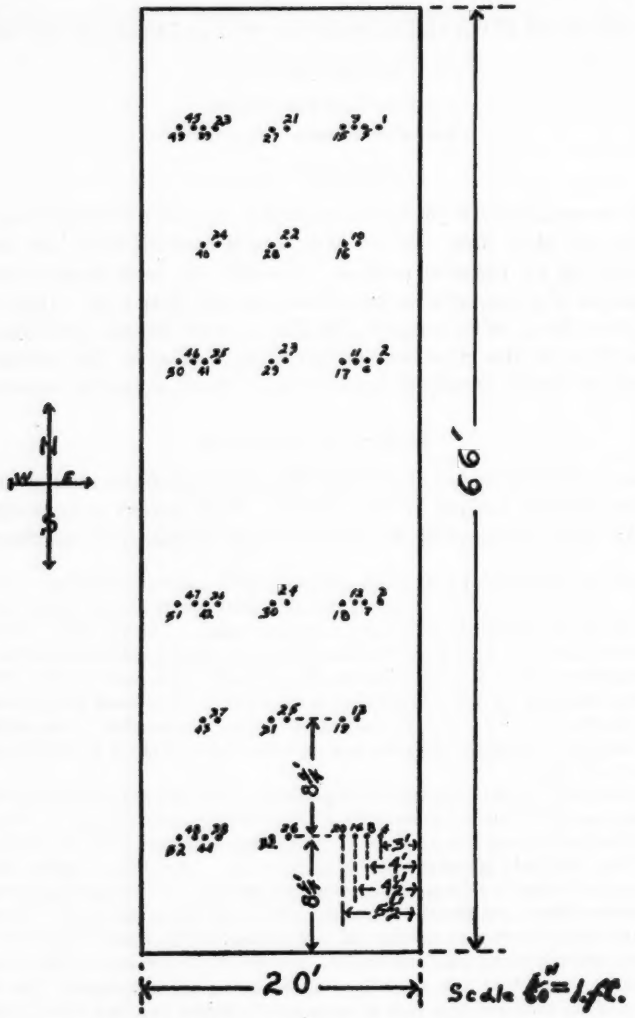

FIG. 1. DIMENSIONS AND LOCATION OF BORINGS ON PLOTS SAMPLED

sampling. Waynick concludes that a composite sample may be considered of value only after the probable error to which it is subject is known, and this can only be determined by the use of a large number of individual samples. Waynick and Sharp (10), in another paper, deter-

mined the probable error in sampling plots for nitrogen and carbon. One hundred samples were taken from each of the two plots sampled. They determined their laboratory error to be 12 pounds of nitrogen per acre when twenty-five determinations were made, and disregarded the laboratory error when one hundred samples were analyzed. They determined the probable error for one field to be ±0.006 per cent, or 240 pounds of nitrogen per acre foot. By knowing the soil variability and probable error for the 100 samples, it is possible to use composite samples for future studies on these plots.

From the variation in the probable errors cited, one would conclude that the results for one area would not be comparable to other areas.[2]

METHODS

Field sampling

Plots A5 and A6 in field VI at the Washington Experiment Station were selected for this work, each being 20 x 66 feet and containing $\frac{1}{33}$ of an acre. The plots were located on the middle to lower portion of a south slope and were considered fairly uniform Palouse silt loam. Since 1899 these two plots have been treated as follows:

A5. Top dressed with 700 pounds of manure in winter after the wheat is up, winter wheat even years and summer fallow odd years.

A6. Plowed early in the spring, tilled well, with winter wheat odd years and summer fallow even years.

Fifty-two samples were taken from each plot as shown in figure 1, each being a core $1\frac{1}{2}$ inches in diameter by $6\frac{2}{3}$ inches deep, and which has been taken as representative of the plowed soil, which is considered as weighing 2,000,000 pounds per acre to this depth. The samples were placed in pint jars, quickly dried and ground to pass a 60-mesh sieve. They were then dried in an oven at 100°C. for five hours and thoroughly mixed.

Chemical analyses

Nitrogen determinations were made by a modified Gunning-Hibbard (1) method, using 5-gram samples with 25 cc. of H_2SO_4, 7–10 gm. of K_2SO_4 and 0.5 gm. of $CuSO_4$. In most cases it was found necessary to digest the samples 7–8 hours to get the best results. Duplicate determinations were made on each sample as a means of checking the laboratory manipulation.

Mathematical calculations

The mathematical treatment will be taken up and discussed briefly, using the data from table 1. This discussion will be applicable to all of the tables.

The mean (m) is obtained for n determinations by adding all of the determinations and dividing by the number of determinations. The standard deviation (σ) is calculated by squaring each deviation from the mean, adding the squares and dividing the sum by the total number of determinations and taking the square root of the quotient (5, p. 428). If the variates are divided into classes, the deviation of each class from the mean is squared and multiplied by the frequency of the variates in that class. The formulas for both methods are as follows:

$$\sigma = \sqrt{\frac{\Sigma\, x^2}{n}} \text{ or } \sigma = \sqrt{\frac{\Sigma\, x^2 f}{n}}$$

[2] Since this paper was completed, the author found an article on the "Variability of Nitrates and Total Nitrogen in Soils," by A. L. Prince, 1923, in Soil Science, v. 15, no. 5. Prince made twenty-five determinations on each of three plots and found that in the case of total nitrogen determinations, the coefficient of variability was about 5.5 per cent for each plot, and the probable error of the mean, about ±0.7 per cent in each case.

where Σx^2 is the sum of the squares of the deviations from the mean, and n the number of variates. In the second formula f signifies *frequency*. Substituting from table 1, we have

$$\sigma = \sqrt{\frac{2{,}069{,}071}{52}} = 199.5$$

The coefficient of variability (C.V.) is the percentage ratio between the standard deviation and the mean (5, p 433) or

$$\frac{\sigma}{M} = \text{C.V.}$$

Substituting from table 1,

$$\frac{199.5}{2528} = 7.88$$

The standard deviation and coefficient of variability only show the spread or variation one may expect in any set of determinations. To show the reliability of any set of observations, the probable error must be calculated.

The probable error (2, p. 39–47) is an arbitrary term and denotes the amount that must be added or subtracted from the mean or observed value to obtain the two extreme figures within which there is an even chance that the true value lies. There is an even chance also that it lies without these limits. As one increases or multiplies the probable error, the chances that the observed result will be within the limiting figures increases.

To obtain the probable error of a single variate (P.E._s) the standard deviation is multiplied by the factor 0.6745 (which is a constant used in statistical calculations), the formula (5, p. 698) being

$$(\text{P.E.}_s) = 0.6745 \times \sigma$$

Substituting from table 1,

$$(\text{P.E.}_s) = 0.6745 \times 199.5 = \pm 134.7$$

To determine the probable error of the mean (P.E._m) of n variates, divide the probable error of a single variate by the square root of the number of variates (5, p. 440) or

$$\text{P.E.}_m = \frac{\text{P.E.}_s}{\sqrt{n}} = \frac{0.6745\,\sigma}{\sqrt{n}}$$

Substituting from table 1,

$$\text{P.E.}_m = \frac{0.6745 \times 199.5}{\sqrt{52}} = \pm 18.7$$

This formula shows the weight which numbers have when calculating probable errors. Figure 2 shows graphically how the probable errors decrease or increase when the number is varied.

In figuring the above constants, it is customary to give the probable error in the constants. To determine the probable error in the standard deviation (P.E._σ) the following formula (5, p. 440) was used.

$$\text{P.E.}_\sigma = \frac{0.6745\,\sigma}{\sqrt{2n}}$$

Substititing from table 1,

$$\text{P E.}_\sigma = \frac{0.6745 \times 199.5}{\sqrt{2 \times 52}} = \pm 13.2$$

Thus the probable error in the standard deviation for plot 5A is ±13.2 pounds, this error being less than the probable error of the mean, because the number of variates is doubled.

When the coefficient of variability is less than 10 per cent, the approximate probable error is found by multiplying the constant 0.6745 by the coefficient of variability (C.V.), and dividing the product by the square root of twice the number of variates (5, p. 441), giving the formula

$$P.E._{(cv)} = \frac{0.6745\ C.V.}{\sqrt{2n}}$$

Substituting from table 1,

$$P.E._{(cv)} = \frac{0.6745 \times 7.88}{\sqrt{2 \times 52}} = \pm 0.52$$

When the coefficient of variability is greater than 10 per cent, the more complicated formula (5, p. 441) is used:

$$P.E._{(cv)} = \frac{0.6745\ C.V.}{\sqrt{2n}} \left[1 + 2\left(\frac{C.V.}{100}\right)^2\right]^{\frac{1}{2}}$$

In this paper the approximate formula was used in all cases.

Besides the above mentioned constants, the probable error in per cent for the mean was also determined by dividing the probable error of the mean by the mean and multiplying by 100. This gives a better comparison of the errors, which in the case of plot 5A and 6A were 0.78 and 0.34 per cent, respectively.

According to Davenport (5, p. 438), the probable error is calculated for any number of variates fixes a range above and below the determined value such that the chances are even that the true value lies within this range. The chances are also even that this value lies outside of the limits set by the probable error, but by multiplying the probable errors of plot 5A and plot 6A by 3, we get 2.34 and 0.99 per cent, respectively, and the chances would be 21 to 1 that the true value would lie within the limits set by the probable errors.

EXPERIMENTAL DATA

The experimental data for the two plots are reported in tables 1 and 2, only the pounds per acre being tabulated, together with the ± pounds deviation from the mean.

It will be noted that the extreme range for the total nitrogen in plot 5A is from 1852 to 2966 pounds, and for plot 6A, 2216 to 2632 pounds, or a difference between the two extremes of 1114 and 416 pounds respectively. This is some indication as to the soil variability that is apt to be encountered in sampling soils.

If but a single sample had been taken to represent either of these plots, it can be readily seen that a very inaccurate estimation of the nitrogen content might have been made. The statistical constants calculated and tabulated at the ends of tables 1 and 2 represent the soil variability (4) in a mathematical way.

It will be noted that the probable error of the mean in plot 5A is 18.7 pounds of nitrogen, or over twice that of plot 6A, which is 8.0 pounds. The standard deviation and coefficient of variability for plot 5A are more than double that of plot 6A, showing the soil of plot 5A to be much more variable than that of plot 6A.

The probable errors as calculated in tables 1 and 2 include both the field and laboratory errors.

LABORATORY ERROR

To determine the laboratory error, twenty-four nitrogen determinations were made on a single sample of soil which was a composite made up by

TABLE 1

Nitrogen as determined for various samples on plot 5A

SAMPLE NUMBER	NITROGEN PER 2,000,000 POUNDS	DEVIATION FROM MEAN ±	SAMPLE NUMBER	NITROGEN PER 2,000,000 POUNDS	DEVIATION FROM MEAN ±
	lbs.	*lbs.*		*lbs.*	*lbs.*
1	2714	186	27	2574	46
2	2622	94	28	2472	56
3	2638	110	29	2508	20
4	2836	308	30	2682	154
5	2659	131	31	2628	100
6	2592	64	32	2697	169
7	2641	113	33	2461	67
8	2690	162	34	2416	112
9	2610	82	35	2377	151
10	2792	264	36	2475	53
11	2569	41	37	2283	245
12	2664	136	38	2450	78
13	2675	147	39	2591	63
14	2821	293	40	2453	75
15	2594	66	41	2221	307
16	2514	14	42	2430	98
17	2527	1	43	2312	216
18	2651	123	44	2471	57
19	2758	230	45	2398	130
20	2966	438	46	2346	182
21	2744	216	47	2206	322
22	2487	41	48	2320	208
23	2548	20	49	2158	370
24	2586	58	50	2271	252
25	2598	70	51	1852	676
26	2726	198	52	2196	322

Mean (M) = 2528 ± 18.7 lbs.
σ = 199.5 ± 13.2 lbs.
C. V. = 7.88 ± .52 per cent
P.E.$_m$ = 0.78 per cent

using 10 gm. of soil from each of the fifty-two samples of plot 6A. The results of these determinations are given in table 3.

The extreme range for the twenty-four determinations was 2368 to 2422 pounds of nitrogen per acre, or a difference between the extremes of 54 pounds. The laboratory error for the twenty-four determinations was very low, being only ±1.6 pounds, or 0.07 per cent of the mean.

THE ACCURACY OBTAINED BY USING A COMPOSITE SAMPLE

In sampling soil, it is often desirable to make a composite sample, thus eliminating a great deal of labor in making chemical analyses. This would be especially true when a large number of plots are to be sampled.

TABLE 2

Nitrogen as determined for various samples on plot 6A

SAMPLE NUMBER	NITROGEN PER 2,000,000 POUNDS	DEVIATION FROM MEAN ±	SAMPLE NUMBER	NITROGEN PER 2,000,000 POUNDS	DEVIATION FROM MEAN ±
	lbs.	*lbs.*		*lbs.*	*lbs.*
1	2360	33	27	2338	55
2	2296	3	28	2334	59
3	2276	116	29	2428	35
4	2352	41	30	2504	111
5	2308	85	31	2418	25
6	2292	101	32	2484	91
7	2282	111	33	2382	11
8	2346	47	34	2364	29
9	2252	141	35	2470	77
10	2298	95	36	2378	15
11	2368	25	37	2400	7
12	2394	1	38	2556	163
13	2218	175	39	2450	57
14	2294	99	40	2322	71
15	2426	33	41	2516	123
16	2374	19	42	2632	239
17	2418	25	43	2350	43
18	2356	37	44	2592	199
19	2216	177	45	2404	11
20	2448	55	46	2480	87
21	2338	55	47	2472	79
22	2364	29	48	2488	95
23	2426	33	49	2416	23
24	2442	49	50	2398	5
25	2362	31	51	2442	49
26	2468	75	52	2434	41

Mean (M) = 2393 ± 8.0 lbs.
σ = 85.7 ± 5.7 lbs.
C. V. = 3.58 ± 0.24 per cent
P.E.$_m$ = 0.34 per cent

Waynick and Sharp (10) have shown in their work that a composite sample is reliable after the soil variability is known. A like comparison was made by the author and the results are given in table 4.

The probable error in the difference is 8.2 pounds, while the difference between the two means is only 1 pound, or $\frac{1}{8}$ of the probable error in the difference, which, according to Reitz and Smith (5, p. 47) would be insignificant. That is, when the difference in two results is less than three times

the probable error in the difference, it is said to be due to random sampling, but as the difference in the two results increases to five or ten times the probable error, it is said to be significant.

From the data given in table 4, it would seem that the use of a composite sample would be justifiable after the soil variability has been determined.

TABLE 3

Nitrogen as determined on a uniform sample

SAMPLE NUMBER	NITROGEN PER 2,000,000 POUNDS	DEVIATION FROM MEAN ±	SAMPLE NUMBER	NITROGEN PER 2,000,000 POUNDS	DEVIATION FROM MEAN ±
	lbs.	*lbs.*		*lbs.*	*lbs.*
1	2382	10	13	2382	10
2	2404	12	14	2398	6
3	2422	30	15	2382	10
4	2392	0	16	2398	6
5	2394	2	17	2388	4
6	2400	8	18	2400	8
7	2382	10	19	2388	4
8	2386	6	20	2382	10
9	2374	18	21	2384	8
10	2398	6	22	2368	24
11	2398	6	23	2388	4
12	2412	20	24	2400	8

Mean (M) $= 2392 \pm 1.6$ lbs.
$\sigma = 11.8 \pm 1.2$ lbs.
C.V. $= 0.48 \pm 0.47$ per cent
P.E.$_m$ $= 0.07$ per cent

TABLE 4

A comparison of nitrogen determinations on a composite sample to those on individual samples

	MEAN POUNDS PER ACRE	PROBABLE ERROR ±
	lbs.	*lbs.*
Determinations on 52 individual samples (from table 2)	2393	8.0
24 determinations on a composite of 52 samples Table 3)	2392	1.6
Difference	1	8.2*

* The probable error in the difference (5) being $\sqrt{(e_1)^2 + (e_2)^2}$ where e designates the probable errors of the two results compared.

NUMBER OF SAMPLES REQUIRED FOR ANY DEGREE OF ACCURACY

In sampling plots it is often desirable to know how many samples should be taken to be within the limits of a certain error. This may be determined mathematically from the results of tables 1 and 2 and tabulated as in table 5.

After the standard deviation is known for any given area, it is possible to vary the number of determinations in the formula

$$P.E._m = \frac{0.6745\,\sigma}{\sqrt{n}}$$

and obtain the different probable errors given in table 5 and shown graphically in figure 2. Or, it is possible to start with a certain probable error and thus determine the number of samples necessary to be within the limits of the required error. For example, suppose we allowed an error of ±18.7

TABLE 5

The total probable error decreases as the number of samples increases

NUMBER OF SAMPLES	PLOT 6A		NUMBER OF SAMPLES	PLOT 5A	
	Probable error of mean ±			Probable error of mean ±	
	lbs.	*per cent*		*lbs.*	*per cent*
1	57.8	2.41	1	134.6	5.32
2	41.3	1.73	2	96.1	3.80
4	28.9	1.20	4	67.3	2.66
8	20.6	0.86	8	48.0	1.90
12	16.5	0.69	12	38.4	1.51
16	14.4	0.60	16	33.6	1.33
20	12.8	0.54	20	29.9	1.17
24	11.8	0.49	24	27.5	1.08
28	10.9	0.46	28	25.4	1.00
36	9.6	0.40	36	22.4	0.88
52	8.0	0.34	52	18.7	0.78

pounds of nitrogen and used the standard deviation from plot 5A, the formula would be

$$18.7 = \frac{0.6745 \times 199.5}{\sqrt{n}}.$$

Solving the equation would give fifty-two samples the number required to give an error of 18.7 pounds of nitrogen. In a like manner, the number of samples for any desired probable error could be calculated. It will be noted that to get a probable error of ±28.9 pounds in plot 6A, four samples should be taken. This will apply only to plots $\frac{1}{8}$ acre in size and quite uniform. In plot 5A four samples would give a probable error of ±67.3 pounds.

The laboratory error decreases or increases just as the total error, as shown in table 6.

The results of the twenty-four determinations made on a single sample in table 3 were used as a basis in calculating the probable laboratory errors, for the varying number of samples in the preceding table. The errors determined in table 6 are graphed in figure 2, and it will be noted that in most work this small error might well be disregarded.

DETERMINATION OF THE FIELD ERROR

In this type of work, there are two errors, namely, field error, which is due to soil variation and random sampling and the laboratory error obtained

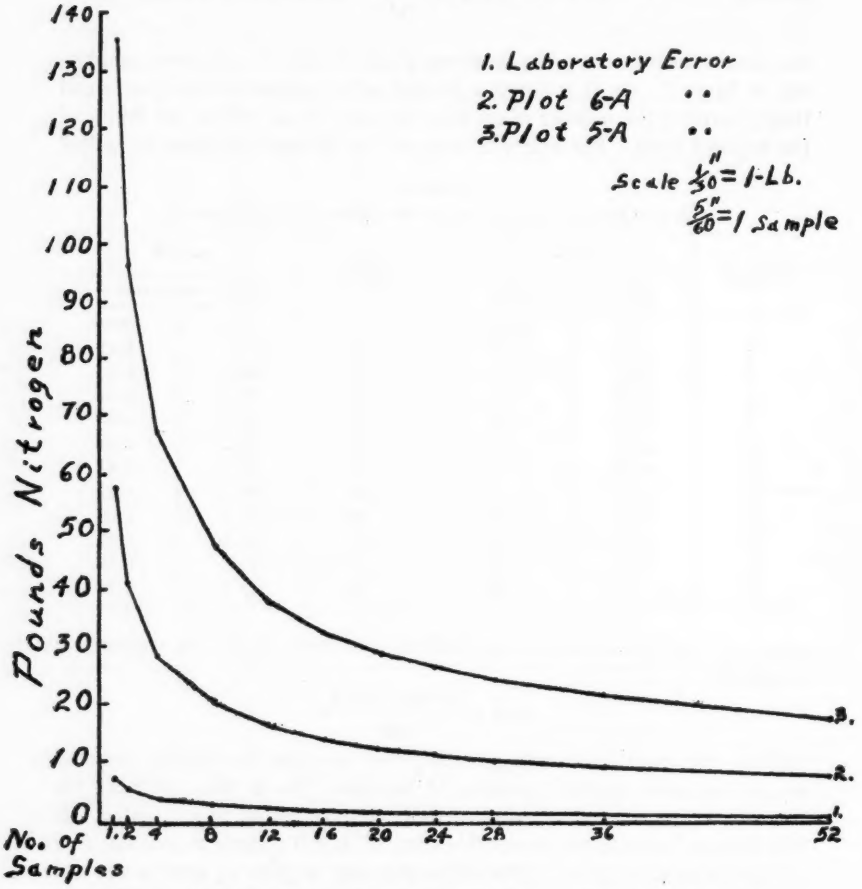

FIG. 2. PROBABLE ERRORS CALCULATED FOR VARYING NUMBERS OF SAMPLES

through manipulation. If we let E represent the total error of the mean with e_1 the laboratory error and e_2 the field error (7), we have

$$E = \sqrt{(e_1)^2 + (e_2)^2}$$

That is, the total error E is not the sum of the two errors as determined, but the square root of the sum of the squares of the separate errors.

In that the values E and e_1 have been determined in tables 1 and 6, the value e_2 can be calculated by substituting in the above formula, as

$$18.7 = \sqrt{(e_2)^2 + (1.1)^2} \text{ or } e_2 = \sqrt{(18.7)^2 - (1.1)^2} = \pm 18.6$$

It is worthy of note that by using the above formula, the field error is practically the same as the total error E, showing a reduction of only 0.1 pound of nitrogen.

TABLE 6

The laboratory error decreases as the number of determinations increases

NUMBER OF DETERMINATIONS	PROBABLE ERROR OF MEAN ±		NUMBER OF DETERMINATIONS	PROBABLE ERROR OF MEAN ±	
	lbs.	*per cent*		*lbs.*	*per cent*
1	7.96	0.33	20	1.77	0.07
2	5.68	0.24	24	1.62	0.07
4	3.99	0.17	28	1.50	0.06
8	2.84	0.12	36	1.32	0.06
12	2.27	0.09	52	1.10	0.05
16	1.99	0.08			

TABLE 7

Summary of the probable errors for plots 5A and 6A

PLOT NUMBER	NUMBER OF SAMPLES	E	e_1	$e_2 = \sqrt{(E)^2 - (e_1)^2}$	
		lbs.	*lbs.*	*lbs.*	*per cent*
5A	52	18.7	1.1	18.6	0.73
6A	52	8.0	1.1	7.9	0.33

TABLE 8

The soil variability by quarters for plots 5A and 6A

QUARTER NUMBER	FOUR SAMPLES AVERAGE NITROGEN PER QUARTER		QUARTER NUMBER	SIX SAMPLES AVERAGE NITROGEN PER QUARTER	
	Plot 5A	Plot 6A		Plot 5A	Plot 6A
	lbs.	*lbs.*		*lbs.*	*lbs.*
1	2293	2424	1	2420	2417
2	2647	2314	2	2601	2356
3	2143	2459	3	2403	2485
4	2701	2314	4	2756	2321

Table 7 summarizes the probable errors for both plots, including the total error E, the laboratory error e_1 and the field error e_2. The values for E and e_1 necessary to calculate the per cent field errors were not tabulated in table 7. However, the same method was used as for pounds nitrogen. It will readily be seen from the data in table 7 that plot 5A is much more variable than 6A as shown by the probable errors in columns 5 and 6. Another point

of interest in table 7 is the field error, e_2, which, in the case of plot 5 A is only 0.1 pound less than the total error, while the field error for plot 6 A is 0.1

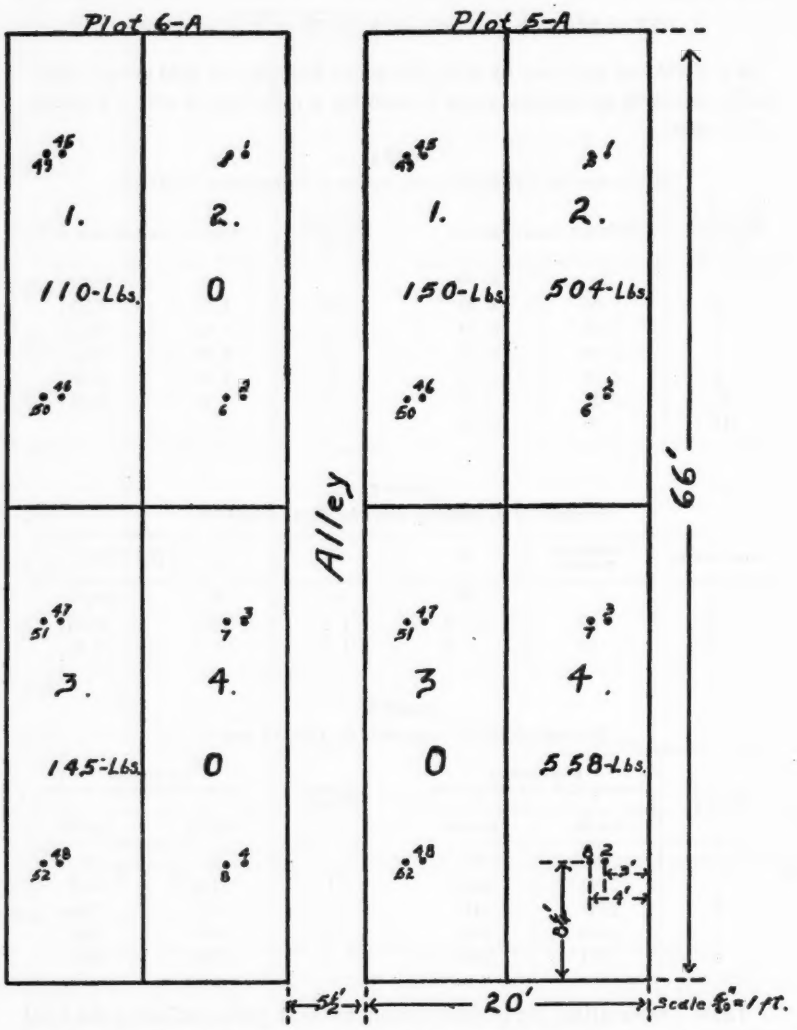

FIG. 3. SHOWING THE SOIL VARIABILITY WHEN FOUR DETERMINATIONS WERE AVERAGED FOR EACH QUARTER

pound less than the total error, showing that in most analytical work of this kind the laboratory error could be disregarded.

ACTUAL SOIL VARIABILITY IN POUNDS PER ACRE

By making a careful study of the two plots 5A and 6A, it was found that one-half of each plot was higher in nitrogen content than the other. This point was of most significance in the manured plot. Thus, to show the actual soil heterogeneity, the plots were divided into quarters and the nitrogen de-

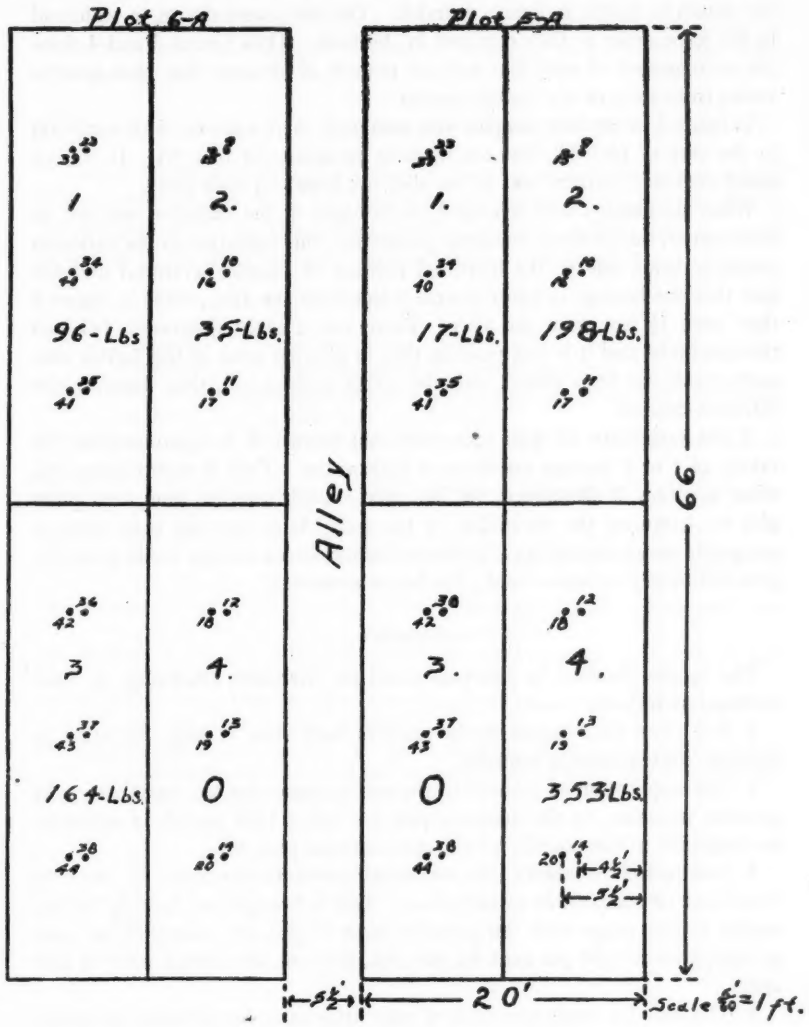

FIG. 4. SHOWING THE SOIL VARIABILITY WHEN SIX DETERMINATIONS WERE AVERAGED FOR EACH QUARTER

termined for each quarter by averaging four and six samples respectively. Table 8 gives the results obtained when the above-mentioned samples were averaged.

The results in the above table were figured on the acre basis. In comparing the quarters of each plot, the one having the lowest mean was taken as zero and subtracted from each of the remaining 3 quarters, giving them the values as shown in figures 3 and 4. The plots were drawn to scale and in the same order as they occurred in the field. Thus figures 3 and 4 show the arrangement of each plot and the pounds of nitrogen that each quarter varies from zero, or the lowest quarter.

In figure 3, where four samples were averaged, there was very little variation in the case of plot 6A, but considerable variation for plot 5A. It will be noted that the quarters next to the alley are lowest in both plots.

When six borings were averaged, as in figure 4, the variation was not so pronounced, but in about the same proportion, this reduction in the variation probably being due to the increased number of samples averaged and the fact that the borings in figure 3 were 3 feet from the alley, while in figure 4 they were 4½ feet from the alley. There was a slight depression between the two plots, and it is just possible that in plowing some of the lighter subsurface soil has been mixed with the richer surface soil, thus lowering the nitrogen content.

If the variability of these plots were not known, it is apparent that the taking of 1 or 2 borings would be of little value. Thus it seems necessary, when accuracy is demanded, to first take enough samples from any given plot to determine the variability of the soil. After this has been done, a composite sample consisting of a determined number of borings would probably give sufficiently accurate results for future analyses.

SUMMARY

The results obtained in this paper and the literature cited may be summarized as follows:

1. Soil plots that appear fairly uniform may show a large variation in nitrogen content among samples.

2 The application of manure to the soil increases the soil variability, the greatest variation or the manured plot 5A, being 1114 pounds of nitrogen, as compared to 416 pounds or the non-manured plot, 6A.

3. Due to soil variability, the statistical constants calculated for one area would not be comparable to any other. This is brought out forcibly by the results in this paper with the probable error of plot 5A being 0.78 per cent as compared to 0.34 per cent for plot 6A, the two plots being only 5½ feet apart.

4. The use of a single sample is of very little value for accuracy in experimental work.

5. A composite sample is of value only after the soil variability has been determined; then enough determinations should be made on the composite to insure a value representative of the soil mass.

6. With careful manipulation, the laboratory error is very low and might well be disregarded, thus making the field error practically the same as the total error.

7. After the soil variability has been determined for any plot, it is possible to calculate the number of samples necessary to be within the limits of a certain error.

CONCLUSION

Thus it seems that to determine the field error in sampling any plot, a fairly large number of samples should be taken and statistical methods applied to the results. The field error determined in this way will be large or small according to the soil variability of the particular plot sampled.

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THE AVAILABLE STATE

J. ALAN MURRAY

University College, Reading, England

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In ordinary cultivated soils the "total phosphoric acid" found by chemical analysis usually amounts to somewhere in the neighborhood of 0.1 per cent of the "air-dried fine earth." According to the conventional methods of computation this represents 2000–3000 pounds of that ingredient per acre; yet applications of 20 or 30 pounds of phosphoric acid in the form of superphosphate may produce a large or even a manifold increase in the crops. This paradoxical result has not been satisfactorily explained. To say that the total phosphoric acid is not all available to the plants is merely to restate the problem since the available state can neither be measured nor defined.

It is commonly held that solution and ionization is a condition precedent to absorption of solids by plants and that the available state must be defined in terms of solubility. Dyer (1) thought that a 1 per cent solution of citric acid acting under arbitrarily defined conditions would serve as a critical reagent to distinguish between that which is available and that which is not. He showed that the productive capacity of soils is more closely correlated with the amount of phosphoric acid soluble in this reagent than with the total phosphoric acid; and he suggested that soils which contain less than 0.01 per cent might be expected to respond to applications of phosphatic manure. This has been confirmed by innumerable observations by others, but probably very few now regard the method as affording a reliable indication of the manurial requirements of soils. In any case it is and has long been recognised that the term "available" in this connection is an unfortunate misnomer, for it is barely conceivable that applications of 20 or 30 pounds of available phosphoric acid should produce the effects observed if ten times that amount were already present in the soil.

It is certain that the returns for manure diminish with each successive unit applied. It is no less certain that they diminish in some regular, definite order. If that order is known, or can be determined, the amount of available phosphoric acid originally present in the soil can be inferred from the increment in the crops produced by applications of different amounts of unquestionably available (water-soluble) phosphates.

Mitscherlich (4), working under controlled conditions, found that the increments produced by successive units of any essential constituent of the plants diminish in geometric progression.

$$\frac{dy}{dx} = K\,(M - y), \text{ or } y = M\,(1 - e^{-kx})$$

Thus, it might be stated that the increment produced by unit quantity of any constituent of which there is a deficiency is proportional to the difference from the maximum. K is a constant; y, the amount of crop corresponding to x units of the fertilizing ingredient, and M the maximum obtainable by addition of any amount of the same.

This thesis has been the subject of much controversy. It has been vigorously attacked (3) and stoutly defended (6). The criticism has developed chiefly along two lines; viz., that the thesis is not exact and that in any case it is not applicable under field conditions. In reply to the former, Mitscherlich points to his experimental results. These are convincing but it has been objected that the simple logarithmic curve corresponding to the formula is not the only one that can be drawn through the points determined. In particular it seems that the true curve may develop a certain reflex bend, resulting from a more or less marked lag in the rate of growth, towards the lower extremity.

To the second objection Mitscherlich rejoins, in effect, that any variation in the experimental conditions would alter the values of the constants K and M but would not otherwise affect the accuracy of the formula which expresses a fundamental law. If the thesis is exact it must apply to experiments in field plots as well as those in flower-pots; but the amount of produce is affected by so many conditions which cannot be controlled in the field that it may be impossible to discern the operation of the law.

The object of the present investigation is, assuming the fundamental truth of Mitscherlich's thesis, to find whether its operation can be traced in the records of field experiments and, if so, to determine from these data the amount of available phosphoric acid originally present in the soil.

The data first examined refer to the growth of lucerne in Australia (2). The experiment was begun in 1916 and the data are as follows:

	Cwt.	*Cwt.*	*Cwt.*
Superphosphate applied (1 cwt. = 112 pounds).....	——	1	2
Average annual hay crop.....	15.25	28.75	37.25

It is fairly obvious that a curve, and only one consistent with the formula, can be drawn to pass through these three points; namely, that in which $M = 51.78$, $K = 0.4614$ and $x = 0.7556$. This value of x is the amount of superphosphate, in hundredweight, equivalent to the "available" phosphoric acid originally present in the soil, i.e., in the plot to which no manure was given. With the addition of 1 cwt. of superphosphate, the amount is increased to $(x + 1)$, with 2 cwt. it is increased to $(x + 2)$ and so on. The grade of manure used is not mentioned in the published account of the experiment, but assum-

ing that it was 30 per cent soluble, these values would correspond to 11.63 pounds, 27.02 pounds and 42.41 pounds of phosphoric acid per acre, respectively.

In figure 1 this curve has been drawn from the point of origin, i.e., from the point at which the amount of available phosphoric acid in the soil is zero. When the amount of manure added is zero, the amount of available phosphoric acid in the soil was, as shown above, 11.63 pounds per acre. The parts of the ordinates shown indicate the amounts of increase produced by each successive unit (cwt.) of manure added. A reflex bend in the curve like that of the broken line would not affect the inference regarding the amount of available phosphoric acid in the soil when no manure was given.

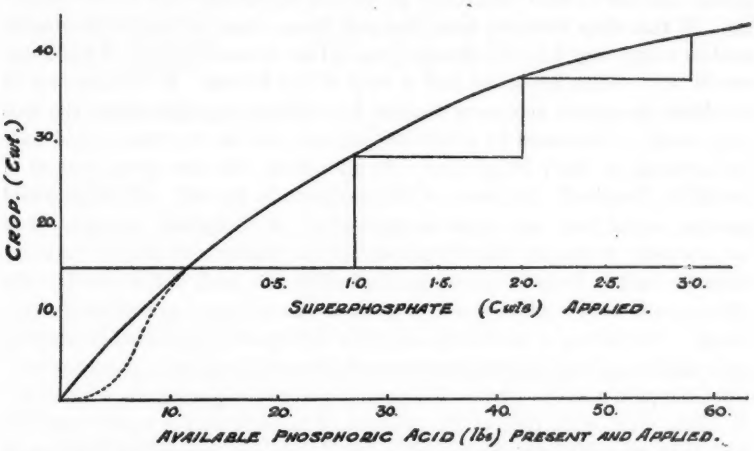

FIG. 1. CURVE SHOWS THE AMOUNT OF CROP CORRESPONDING TO THE QUANTITY OF AVAILABLE P_2O_5 IN THE SOIL AND THE INCREASE PRODUCED BY EACH UNIT OF FERTILIZER APPLIED

The hay was valued at £3 10/- per ton and the superphosphate cost 7/- per cwt. The quantity of the latter might therefore have been increased advantageously to about 5 cwt. This represents nearly 77 pounds of phosphoric acid in addition to the 11.63 pounds present originally, and would have increased the crop to 48 cwt. of hay per acre. In the author's opinion the cost of spreading should have been added to the price of the manure and the cost of harvesting should have been deducted from the value of the crop. If that were done it would not be profitable to use more than about 4 cwt. of superphosphate. But taking the figures as they stand it appears that under similar conditions of climate and soil it would not be necessary or advantageous to apply phosphatic manures to lucerne on soils that contain more than about 85 pounds per acre of phosphoric acid actually available to the plants.

If the conclusions drawn are exact the effects of the manure are readily intelligible. In the circumstances considered a ton of lucerne hay would be produced if the soil contained 15.5 pounds of available phosphoric acid. By the application of 1 cwt. of superphosphate the amount of available phosphoric acid in the soil is doubled, by the application of 2 cwt. it is trebled and so on. The crops would not, of course, be increased in the same ratio but, if they were not limited by deficiency in other conditions, large increments would naturally be expected from such manifold increments in the amount of available phosphoric acid.

These considerations also help to establish a connection between the chemical composition of crops and their manurial requirements. The apparent absence of such connection at present is generally felt to be disturbing. A root crop removes from the soil about twice as much phosphoric acid as a hay crop, i.e., the amount required for an average crop of the latter would only suffice for about half a crop of the former. If this amount of available phosphoric acid were doubled by applying superphosphate the root crop would be increased by about 100 per cent but the hay crop would only be increased by (say) 10 per cent. At all events, with any given amount of available phosphoric acid short of the maximum in the soil, root crops would respond better than hay crops to applications of phosphatic manure. It is not necessary to assume that the grasses have a greater assimilative capacity than the roots. It is of course, quite possible that such is the case but the difference, if any, is perhaps not so great as has hitherto been generally supposed. The failure of chemical analysis to distinguish with certainty between soils which require phosphatic manure and those which do not is also accounted for. Suppose a soil to contain 275 pounds per acre of phosphoric acid soluble in dilute citric acid, that of this amount 25 pounds were actually available and that an additional 50 pounds—say 4 cwt. of superphosphate—were applied; the amount of citric soluble phosphoric acid would be only 0.011 per cent in the former case and 0.013 per cent in the latter. Such a small difference would generally be regarded as negligible though it would actually mean a threefold increase in the amount of available phosphoric acid. In short, chemical analysis has failed because, generally, the percentages of total phosphoric acid, citric soluble phosphoric acid, available phosphoric acid in productive soils and available phosphoric acid in unproductive soils are respectively of the order of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}.

Additional evidence in support of these important inferences has been obtained from the records of an experiment carried out by the author in the years 1893–1896 inclusive. The objects of this experiment were to determine the maximum amount of phosphatic manure that could be profitably applied, with and without nitrogen, to hay crops on a flat alluvial meadow at Falcondale in Cardiganshire, and to compare superphosphate with basic slag as a source of phosphate, and nitrate of soda with sulfate of ammonia as a source of nitrogen. The quantities of manure were adjusted to represent

TABLE 1

Weight (cwt.) of hay from each plot, the maximum (M), constant (K) and amount of available phosphates (X) originally present in the soil

Treatment	EXPERIMENTAL DATA: Superphosphate A. None	B. 1 cwt.	C. 2 cwt.	D. 3 cwt.	INFERENTIAL DATA: M	K	x
	cwt.	cwt.	cwt.	cwt.			
1893							
I. No nitrogen	4.86	5.93	6.63	*7.11** / 7.14	8.00	0.417	2.264
II. 112 pounds $NaNO_3$	10.00	11.57	11.79	*11.84* / 12.21	11.85	1.888	0.984
III. 224 pounds $NaNO_3$	13.50	18.43	18.58	18.58			
IV. 90 lbs. $(NH_4)_2SO_4$	7.97	10.58	10.58	8.71			
V. 180 pounds $(NH_4)_2SO_4$	10.92	12.06	12.93	*13.21* / 15.00	15.55	0.238	4.285
1894							
I. No nitrogen	21.72	*26.31* / 18.07	27.72	28.15	28.36	1.176	1.235
II. 112 pounds $NaNO_3$	24.43	30.36	34.00	*36.21* / 35.00	39.74	0.490	1.946
III. 224 pounds $NaNO_3$	*29.42* / 25.28	37.71	40.86	42.14	42.79	0.968	1.202
IV. 90 pounds $(NH_4)_2SO_4$	25.43	31.15	32.36	*32.66* / 31.72	32.74	1.526	0.983
V. 180 pounds $(NH_4)_2SO_4$	34.15	38.00	39.14	*39.38* / 39.43	39.49	1.276	1.568
1895							
I. No nitrogen	8.84	11.71	*13.81* / 15.86	15.29	19.36	0.318	1.929
II. 112 pounds $NaNO_3$	10.15	15.86	18.28	*19.31* / 17.43	20.05	0.860	0.821
III. 224 pounds $NaNO_3$	14.00	16.71	*18.18* / 21.57	19.00	20.00	0.601	2.004
IV. 90 pounds $(NH_4)_2SO_4$	11.14	13.00	14.42	*15.52* / 12.57	19.09	0.267	3.286
V. 180 pounds $(NH_4)_2SO_4$	14.57	15.71	*16.10* / 13.28	16.14	16.29	1.087	2.068
1896							
I. No nitrogen	14.63	17.93	19.93	*21.13* / 22.64	23.00	0.501	2.017
II. 112 pounds $NaNO_3$	18.58	21.64	*23.98* / 26.07	25.78	31.81	0.263	3.332
III. 224 pounds $NaNO_3$	24.72	27.79	*29.45* / 27.79	30.36	31.42	0.613	2.522
IV. 90 pounds $(NH_4)_2SO_4$	20.35	22.51	23.29	*23.49* / 19.22	23.62	1.081	1.830
V. 180 pounds $(NH_4)_2SO_4$	29.85	27.94	23.08	26.94			

* The figures in italics are the amounts of crop calculated by the formula for the plots not involved in the determination of M, K and X.

equal amounts of phosphoric acid and equal amounts of nitrogen from the sources of these ingredients, respectively. The plan of the experiment, as far as the superphosphate is concerned, will be sufficiently obvious from table 1.

It will be seen that there was a control plot and three others to which different quantities of superphosphate were applied in each case. This gives altogether four points on each curve and the values of M, K and x can be determined from any three of them, i.e., in four different ways. If the data were absolutely (mathematically) consistent precisely the same results would, of course, be obtained in each case. Such perfection is not, however, attainable in matters of this kind and the choice of alternatives was at first embarrassing. It is obvious, however, that the values obtained from the first three plots (A, B, and C) are generally to be preferred because in many cases the increase produced by the third hundredweight of superphosphate (plot D) brings the crop very near to the attainable maximum and any variations due to experimental error and uncontrolled causes affect the values (M, K and x) to a much greater extent than when these are calculated from data in which the margin is wider.

Irregularities arising from these causes are not, of course confined to plot D but presumably affect the crops on all the plots in greater or less degree. In some cases they are easily detected. For example when the produce from one of the manured plots is less than that of the control the result is clearly a discrepancy. Or, again, if the amounts of increase produced by successive units of manure applied are not in the order of diminishing quantity the data must be regarded as untrustworthy; and, in such cases, it is often easy to decide which plot is out of order. Altogether, three plots, viz., plots III and IV in 1893, and plot V in 1896 had to be discarded for these reasons; the irregularities being such that no inference could be drawn. In all the other cases the values of M, K, and x were determined from the three plots that seemed most consistent, and the amount of the crop calculated by the formula for the fourth plot, which was not involved in the determination of M, K and x, is shown in italics above the result actually obtained.

The irregularities are naturally greater and of more frequent occurrence in abnormal seasons. Unfortunately, only in one year, 1894, out of the four could the season be described as normal. In that year, 21 cwt. of hay was obtained from the control plot and nearly twice as much from some of those to which manures were applied. The first year of the experiment, 1893, was characterized by prolonged drought and only about a quarter crop was produced. In 1895 and 1896 the drought was not so severe and in these two seasons the crops amounted to about half and three-quarters of the normal yield, respectively.

Keeping these facts in mind the figures in the table may be left to tell their own tale. The two points to which attention should be more particularly directed are (a) comparison of the observed results (in ordinary type) with those calculated (in italics above) and (b) the value of x which is the amount

of superphosphate equivalent to the available phosphoric acid originally present in the soil. To determine the latter, it has been said, is the principal object of the investigation; the former is of impotance only as a test of the reliability of the estimate. To facilitate comparison the condensed results are presented in table 2.

The uniformity of the figures in the last column is, in the circumstances, quite remarkable. It signifies, apparently, that the amount of available phosphoric acid, as determined by this method, is not affected by the presence or absence of nitrogenous manures or by the kind or quantity of the same if used. The figures in the bottom row are not uniform; on the contrary, if the results for 1893 be ignored, they exhibit a progressive increase from year to year which probably represents the unexhausted residue of the manure applied in the preceding years. If this explanation be accepted and allowance made for it, the approximate uniformity thus inferred may be held to signify that the amount of available phosphoric acid as determined by this method, is not affected by drought or moisture except in so far as drought tends to produce irregularities which obscure the result.

TABLE 2

Available phosphoric acid in terms of superphosphate originally present in the soil (i.e., x)

	1893	1894	1895	1896	MEAN
	cwt.	*cwt.*	*cwt.*	*cwt.*	*cwt.*
No nitrogen	2.246	1.235	1.929	2.917	1.857
112 pounds $NaNO_3$	0.984	1.946	0.821	3.332	1.771
224 pounds $NaNO_3$		1.202	2.004	2.522	1.909
90 pounds $(NH_4)_2SO_4$		0.983	3.286	1.830	1.700
180 pounds $(NH_4)_2SO_4$	4.285	1.568	2.068		2.604
Mean	2.505	1.387	2.022	2.425	1.968

The mean of all the results is 2.015 ± 0.6 and in view of the magnitude of the probable error perhaps no great confidence can be reposed in the conclusions. It may be held that the figures represent no more than the irregularities due to the exceptional character of the seasons and that they have fortuitously combined to produce precisely the results to be expected. It is to be observed, however, that the season of 1894 was a normal one and that the results obtained in that year exhibit a satisfactory degree of consistency. Arguments founded on the mean for that year alone may therefore bear more stress than those above referred to.

At all events, notwithstanding the variations in detail, the important fact remains that the results are all essentially of the same order. They afford fairly conclusive evidence that the amount of available phosphoric acid originally present in this soil is equal to that contained in 1–2 cwt. of superphosphate (25 per cent soluble), i.e., in round numbers, about 20 pounds of phosphoric acid per acre, or about twice as much as was found in the case

of the more responsive Australian soil. By application of 1, 2, and 3 cwt. of superphosphate this would be increased to 33 pounds, 46 pounds, and 59 pounds, respectively. The last mentioned quantity, it will be seen, represents a threefold increase in the amount of available phosphoric acid.

A law of diminishing returns implies that the smaller the amount of any available plant food in the soil the larger will be the percentage of it removed by the crops, but that under no circumstances will the whole of it be utilized. The crop of 22 cwt. of hay obtained from the unmanured plot in 1894 would remove only 8 or 9 pounds of phosphoric acid out of the 20 pounds available. The crop of 5 cwt. obtained in 1893 would remove only about 2 pounds, i.e., about one-tenth of the amount available. It is remarkable that, in these circumstances, the crop in 1893 should have been increased by nearly 20 per cent by the application of 1 cwt. of superphosphate. This quantity of manure, itself, contained 13 pounds of available (water soluble) phosphoric acid, i.e., over six times as much as was removed by the crop, but a further increase was produced by the second hundredweight and a still further increase by the third. It is necessary, therefore, to inquire precisely what meaning is to be attached to the term available in this connection.

If it be true that solution and ionization is a condition precedent to absorption of salts by plants, only that portion of the phosphoric acid which is actually dissolved can be regarded as in any real sense available. In natural soils the amount of phosphoric acid in solution at any given time is relatively small. The average amount found by Voelcker (8) in the drainage water from Broadbalk field at Rothamsted was 0.93 parts per million. Reckoning the quantity of water in the soil as a fourth of the dry weight, this would represent only about 0.2 pound per acre. Whitney and Cameron (9) found from five to eight times as much in the soil solutions which they obtained by centrifugal methods. By the same method of computation this represents only from 1 to 1.5 pound of phosphoric acid in solution per acre. The film-water solution which cannot be extracted by this means is probably still more concentrated but there is no good reason to suppose that at any given time the amount of phosphoric acid in solution is as much as is removed in the crops during their period of growth.

But even if such were the case that quantity would not suffice for the requirements of the plants. The rate of diffusion would be reduced as the ions were withdrawn from the solution and infinite time would be required to complete the process. It is evident, therefore, that there must be a reserve of undissolved material able to pass more or less rapidly into solution and replenish the supply of ions. The rate at which this occurs will depend upon (a) the temperature and concentration of the existing solution, (b) the amount of carbon dioxide in the solution and therefore to some extent upon the amount of organic matter in the soil and the freedom of access of air to the same, (c) the state of combination in which the phosphoric acid is present and (d) upon the extent of surface exposed. Under like conditions in other

respects the rate at which ions removed by growing plants are replaced will be governed by the last mentioned condition.

The extent of surface varies inversely as the size (diameter) of the particles. The curve (fig. 2) by which this relationship is expressed is "L" shaped, i.e., it changes somewhat abruptly from the approximately horizontal to the approximately vertical direction as it approaches the zero point. The region of the bend probably corresponds to a critical stage similar to, if not identical with, that which marks the transition to the colloid state. It may be supposed, therefore, that the corner is turned by a range of particles of the order of 10^{-4} mm. in diameter and that any increase in the number of particles of smaller size will greatly accelerate the rate at which ions removed from the solution are replaced therein.

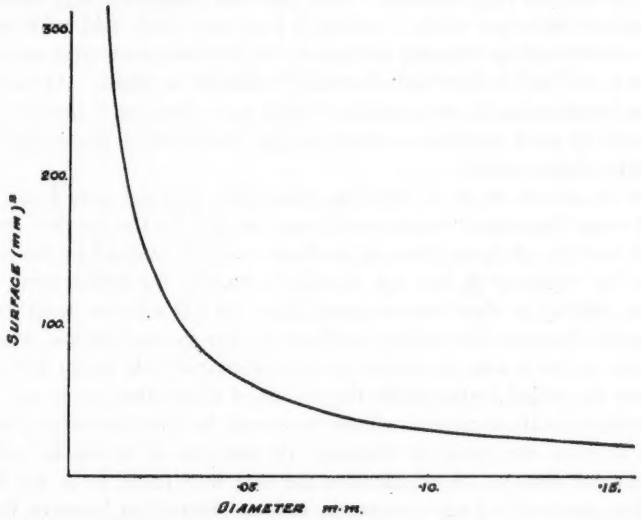

FIG. 2. THE CURVE ILLUSTRATES THE INCREASE IN TOTAL SURFACE DUE TO FINE SUBDIVISION OF PARTICLES. THE REGION OF THE BEND PROBABLY CORRESPONDS TO A CRITICAL STAGE BETWEEN THE AVAILABLE AND NON-AVAILABLE STATES

When superphosphate is applied to soils the soluble phosphoric acid does not remain in that state but is converted into other compounds and solid particles are formed. It is not necessary to suppose that these particles are all of the same kind, or all of the same size, but only that a larger or smaller proportion of them are below the critical stage and therefore subject to comparatively rapid solution. When the number of particles of this degree of fineness becomes so large that the ions can be replaced as rapidly as they are removed any further increase would not affect the amount of the crop.

It has been shown that the soil now under investigation contains about 20 pounds per acre of available phosphoric acid and it is clear, from the method employed, that this means merely that the conditions prevailing in the soil are precisely those which would result from the application of superphosphate containing that amount of soluble phosphoric acid if none of that ingredient were originally present.

The results obtained, especially in 1893, cannot be explained on the assumption that the P_2O_5 applied as superphosphate remains soluble or in any real sense available to the plant; and to call that originally present in the soil available is to confuse the issue. The point to be emphasized is that it is essentially of the same character as that derived from superphosphate after it has reacted with the soil constituents. The term "superfine" appears aptly to describe this condition. This superfine phosphoric acid should not be confused with that which is soluble in 1 per cent citric acid. The term is to be understood as referring exclusively to the phosphoric acid which was earlier in this article described as actually available to plants. At that stage of the investigation it was considered right so to describe it because it was obviously as much available to plants as the water-soluble phosphate in the superphosphate applied.

The 20 pounds or so of superfine phosphoric acid per acre found in the soil is more than would be removed from the land by the heaviest crop obtained in 1924. It is ten times as much as would be removed by the crops of 1893; but evidently it was not enough to provide for replacement of the ions as quickly as they were removed from the soil solution in either case. It appears therefore that neither moisture nor nitrogenous manures accelerate the rate at which ions are abstracted to any considerable extent but merely prolong the period during which the process of abstraction continues. Such, at all events, is the hypothesis offered to account for the phenomena observed.

An attempt was made to determine the amount of "available" nitrogen in the same way but on examination the data were found to be too limited and irregular to afford any trustworthy results. Since then, however, Prescott (7) has published an account of experiments on the application of nitrate of soda and sulfate of ammonia to maize crops in Egypt in which the plots were more numerous and very consistent results were obtained. These, together with the inferential data, are reproduced below. As before, the figures in italics are the amounts of crop calculated for the plots not involved in the determination of M, K and x.

	KILOGRAMS PER ACRE					
Nitrate of soda	....	100	150	200	300	400
Nitrogen in same	....	15.1	22.7	30.3	45.4	60.5
Crop (dry ears)	1610	2276	2410 *2524*	2730	2936 *3042*	2910 *3253*

$$M = 3708; K = 0.382; \frac{x}{100} = 1.49$$

	KILOGRAMS PER ACRE					
Sulfate of ammonia.....	....	75	150	200	250	300
Nitrogen in same.....	....	15.3	30.7	40.9	51.1	61.3
Crop (dry ears).....	1610	2304	2670	2950	2930	3050
				2810	*2902*	*2936*

$$M = 3075; K = 0.6421; \frac{x}{100} = 1.155$$

For convenience in calculation, x was found in terms of hundreds of kilograms. The amount of "available" nitrogen originally present in the soil was therefore equivalent to:

149 kgm. nitrate of soda = 22.5 kgm. nitrogen per acre
115.5 kgm. sulfate of ammonia = 23.6 kgm. nitrogen per acre

The large number of plots (6 in each case), the consistency of the data and the close agreement of the results obtained with two different kinds of manure tend to inspire confidence not only in this particular experiment but also in the method of investigation when subject to controlled conditions.

The 50 pounds of nitrogen per acre found to be originally present is not the total nitrogen in the soil. It is the amount of nitrogen potent in fertilizing effects as that in equivalent quantity of nitrate of soda or sulfate of ammonia. These substances are soluble in water and the nitrogen they contain is not converted into insoluble compounds by reaction with other soil constituents. It is reasonable to suppose therefore that this quantity (50 pounds per acre) of nitrogen was present in the form of soluble compounds and was truly available to the plants. As the yield of straw is not mentioned it is impossible to estimate exactly how much nitrogen was removed from each plot, but the amount was probably not far short of the total originally present and added in the manure.

In England the conditions are, of course, very different. So far as can be ascertained from the data previously given in the table the amount of "available" nitrogen originally present in the soil was only about 15 pounds per acre. Judging by the amount of nitrogen found in the drainage water at Rothamsted this is three or four times as much as would be present in the soil solution at any one time. In any case it is less than a third of the amount removed in the crop in 1894 from plot I D which received no nitrogenous manure. This implies the conversion of a large amount of nitrogen from an insoluble to a soluble state during the period of growth and is therefore in agreement with prevailing ideas on the subject. As the rate at which these changes occur depends so largely upon temperature and moisture the amount of available nitrogen present at any one time must be regarded as more or less

fortuitous. It does not appear therefore that this method of investigation is likely to prove of much value in regard to nitrogen as far as this climate is concerned.

The close correspondence of the results obtained under carefully controlled conditions, however, encourages the hope that it may be found possible to employ the method in the laboratory to determine the manurial requirements of soils as regards the mineral elements. The result of such experiments, if found to agree substantially with those of field trials, would furnish a valuable standard of comparison for any purely chemical methods which may be evolved in the future. It is to be expected that if the aim is once clearly established, suitable chemical methods will be discovered. It is already clear that such methods must be applied to samples of the soil taken *in situ*. To dry the soil, disintegrate it and eliminate the larger particles, euphemistically described as stones, is indefensible. Results expressed as percentages of the "air-dried fine earth" are useless.

SUMMARY

It is suggested that the amount of available phosphoric acid in soils may be found from the rate at which the amounts of increase produced by application of different quantities of water soluble phosphates diminish.

The amount so found in an Australian soil was about 11 pounds per acre and it is claimed that this explains the effect of the manure, accounts for the difference in the response of grass and root crops and for the failure of chemical analysis to determine with certainty the manurial requirements of soils.

In an English meadow soil the amount of available phosphoric acid found was, on the average of four years trial, about 20 pounds per acre. Much the same results were obtained in dry and wet seasons and they were not affected to any considerable extent by the applications of nitrogenous manures. In the third and fourth year the data bear evidence of the residual effects of the manure applied in the preceding years.

It is pointed out that water soluble phosphates revert to insoluble forms by reaction with other soil constituents, that the rate at which they are redissolved must depend largely on the size of the solid particles formed and that there must be a critical stage.

It is considered that only the phosphoric acid in solution is actually available to plants and that the amount of phosphates found by this method would be more accurately described as superfine phosphates because it is as potent as that applied in the form of superphosphate and must be presumed to be in an equally fine state of division.

The hypothesis offered to account for the phenomena observed is that ions are removed from the soil solution by growing plants and are replaced by the passing into solution of the most minute solid particles. The effect of the manure is to increase the number of these particles. When they are

so numerous that the ions are replaced as rapidly as they are abstracted the maximum effect of the ingredient applied is produced and the crop will not be further increased by addition of any larger quantity.

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